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Brit. J. Ophthal. (1954) **38**, 445.

COMMUNICATIONS

OCULAR HISTOLOGY IN PREMATURE INFANTS WITH REFERENCE TO RETROLENTAL FIBROPLASIA

BY

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In the years following the first description of retrolental fibroplasia by Terry (1942), the reported incidence of the disease rose alarmingly and it was soon considered by many to be the most common cause of blindness in children under school age. Much thought and study have been given to the aetiology of retrolental fibroplasia and almost every aspect of pregnancy and premature baby management has come under close scrutiny. About the time this investigation was planned, interest was focused on the question of pre-natal versus post-natal origin; was some predisposing condition or nucleus of disease present at birth? Whilst there was little clinical evidence of congenital affection, some investigators (Heath, 1950; Reese and Blodi, 1951; Reese, and others, 1952) described pathological changes in eyes obtained from premature infants at or within the first day or two of birth.

It was, therefore, decided to examine the eyes from a significant number of premature infants in search of early signs of retrolental fibroplasia and of physiological or pathological conditions which might predispose to this disease.

Review of the Literature

Previous reports on such material, reviewed in chronological order, have been carried out by Heath (1950) who stated that forty eyes of foetuses of 4 to 9 months' gestation, studied microscopically, showed differentiation consistent with gestational ages. None showed retrolental fibroplasia or significant retinal changes. The retina, according to its degree of prematurity, showed loose coaptation and the usual variable anterior insertion. An occasional well-contained haemorrhage was seen in the retina. No excess of vascular tissue and no disruption, heavy staining, or marked oedema of retinal structure were seen, and no vitreous haemorrhage was noted.

Reese and Blodi (1951) examined the eyes of 93 premature infants who were either stillborn or died shortly after birth. Fourteen sets were too macerated for useful observation; of the remaining 79 sets, 21 were from stillbirths. They found neo-vascularization of the retina spreading into the vitreous in both eyes of one case in which the birth weight was 1,950 g., and in another case they found new vessels on the surface of the retina. These cases would now be diagnosed as an early stage of retrolental fibroplasia. An inflammatory process of the uvea was found in 18 per cent., usually only in the choroid. Occasionally cells were seen in

the vitreous, and, in one case particularly, around the hyaloid system. The degree of infiltration was not specified, but that shown in their illustration was of considerable extent. A large retinal haemorrhage in the region of the ora was noted in 14 per cent. of cases, and small haemorrhages elsewhere were extremely common. Massive congestion of the retina and uvea occurred in 14 per cent. of cases. The authors commented that such conditions can be due to *post-mortem* change in association with maceration, although marked dilatation was also seen in eyes showing no other *post-mortem* change.

Dixon and Paul (1951), who examined twenty premature and ten full-term infants, concluded that factors which interfere with development may prevent normal adherence between the layers of the optic cup. Heath (1951) in a second paper on the examination of eyes from an unspecified number of stillborn premature infants ranging from 3 months to full term (it is not clear how many of these were included in his first report), concluded that some oedema and occasional retinal haemorrhages occurred, but found no significant herniating neo-vascular tissue. He pointed out that in the oral zone, capillary structure was relatively sparse and the endothelial cells small and less deeply staining, that the internal limiting membrane stopped near the point at which the retinal loop began, and that Müller's fibres were absent. The retinae of premature infants in general were also inserted well anteriorly, sometimes on the pars plana and ciliary body.

Friedenwald and others (1951) examined premature stillborn infants' eyes and found sprouting capillaries and accompanying " glial " proliferations in the retinae, but these had an orderly arrangement and the capillary whorls likened to glomerular tufts were essentially lacking in the normal developing process. Reese and others (1952) describe the examination of 97 premature infants whose birthweights ranged from 300 to 2,500 g. (It is not clear how many of these are included in the material for their first paper). They found two cases of early retrolental fibroplasia: one stillbirth of 1,950 g. with a mass of neovascular tissue in the vitreous—described in their earlier series—, and an infant of unknown birth weight who died within the first 48 hours of life, showing increased " glial " and endothelial cell and capillary formation in the equatorial region, and an apparent break-through into the vitreous in an area of folded retina in one eye. Examining further the relationship of retinal and choroidal hyperaemia to *post-mortem* change, they found that the two ran parallel, and, as hyperaemia was not more common in infants of low birth weight, concluded it could not be an early stage of retrolental fibroplasia. Absence or detachment of the internal limiting membrane in the region of the ora—a common finding—was similarly considered to be related to maceration and not to birth weight.

Excluding foetuses (*i.e.*, infants with a birth weight of under 300 g.), retinal haemorrhages were found in 25 per cent. of cases and situated in the oral region in 10 per cent. Cells in the uveal tract, previously regarded as inflammatory, are identified by these authors as foci of extra-medullary haemopoiesis and marked for further investigation. Incidental congenital anomalies, not common in this series, included retrobulbar cyst with microphthalmos, retinal dysplasia, retinal rosettes, iris cysts, retinal tissue in the pars plana, and accumulations of large polygonal cells with eosinophilic granules on the ciliary epithelium.

To summarize the literature on this subject, no evidence was found of vascular proliferation in " normal " prematures except for the case described

by Reese and others (1952) of an infant of unknown birth weight who died aged 48 hours and showed signs of early retrolental fibroplasia, and the statement by Heath (1951), describing the primary retinal disease which occurs in the anterior differentiated retina at its insertion, that these "dissociating elements (vascular dilatation, oedema, haemorrhage, and endothelial proliferation) could be found within a day of birth", though they might not develop until weeks later.

It is generally agreed that loose retinal coaptation, anterior retinal redundancy, and variable anterior insertion are premature ocular characteristics. Dixon and Paul (1951) stressed the development of adherence between the layers of the optic cup and suggested that some factor affecting this process was of importance in retrolental fibroplasia. Wolff (1950) had suggested that this factor might be some noxious stimulus which reached the embryo in the first few months of intra-uterine life causing the retina to remain unattached, so that true detachment ensued, followed by mild uveitis and retrolental fibroplasia, which was thus due to the formation of a cyclitic membrane.

Inflammatory processes of the uvea are described as occurring in 18 per cent. of cases (Reese and Blodi, 1951), and the theory has been put forward that this represents extramedullary haemopoiesis.

Defects of the internal limiting membrane and Müller's fibres in the oral zone were described by Heath (1950) and Reese and Blodi (1951), but on further investigation Reese and others (1952) showed these changes to be related to maceration and not to birth weight. Retinal and choroidal hyperaemia were similarly shown to run parallel to *post-mortem* change.

Heath (1950) considered some degree of retinal oedema and occasional haemorrhage to be commonplace. Reese and others (1952) found retinal haemorrhages occurring in 25 per cent. of cases with birth weight of over 300 g., and stated that in 10 per cent. the haemorrhage was considerable and situated in the region of the ora.

Purpose of Present Study

In this study, attention will be chiefly directed towards retinal vascularization and coaptation. By comparing the characteristics of cases showing variations of these processes with the average characteristics of the eyes from one hundred premature babies, an attempt will be made to assess the significance of these variations with particular reference to retrolental fibroplasia.

Material

The material was obtained in the British Isles; 109 pairs of eyes were received in response to a Medical Research Council appeal. From these were excluded two cases of definite early retrolental fibroplasia, two cases of anencephaly, one case of intra-uterine keratouveitis, and four cases in which, owing to advanced *post-mortem* degeneration or maceration, efficient examination was not considered possible.

The material reported on was made up as follows:

(1) Stillborn premature babies	13	
(2) Premature babies living less than 1 day	26	
(3) Premature babies living less than 1 week	47	100
(4) Premature babies living more than 1 week	9	
(5) Premature babies of unknown age or weight	5	

The degree of *post-mortem* degeneration encountered varied enormously depending on the time between death and fixation. In a few cases formalin had been injected intra-ocularly immediately after death.

Method

On reception the eyes were opened by horizontal section and any macroscopic changes noted; they were then dehydrated and embedded in celloidin, and two to three sections were cut (through the optic disc unless otherwise indicated) and stained with haematoxylin and eosin and with periodic acid-Schiff stain.

Later a number of unfixed eyes were injected with Indian ink *via* the central retinal vessels, frozen to facilitate removal of the vitreous, and fixed in 10 per cent. formol saline. They were then opened coronally in the oral region; the lens and vitreous were removed, and the retinae examined *in situ*, before removal, staining, and mounting as a flat preparation.

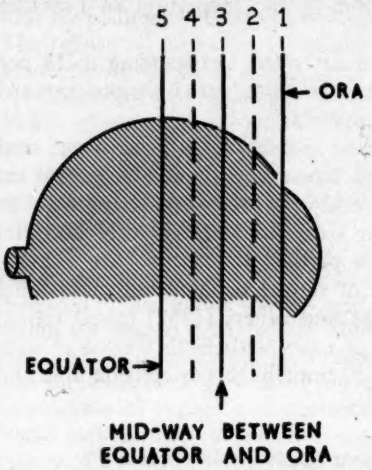

FIG. 1.—Method of corneal sectioning.

A comparable series of stillbirths and live births of ages ranging from 2 to 12 days were sectioned coronally and sections stained with haematoxylin and eosin in the areas numbered 1–2–3–4–5 in Fig. 1. These positions were estimated arbitrarily by the microtomist.

Results and Conclusions

Retinal Vascularization.—The normal degree of vascularity and quantity of vaso-formative tissue in the immature retina appeared to vary widely and it became apparent that folding, section thickness, and *post-mortem* change affected microscopic appearances profoundly. However, in thirteen cases it was decided that deviation from the normal, based on examination of 100 eyes, was noteworthy. The abnormal changes observed comprised:

(*a*) diffuse thickening of the nerve fibre layer due to an increase in endothelial and " glial " cells, since shown to be mesenchymal in origin (Ashton, 1954; Serpell, 1954).

(*b*) glomerular whorls, columns, and solid masses, composed chiefly of small spindle-shaped hyperchromatic mesenchymal cells and to a lesser degree of larger, more differentiated, palely-staining endothelial cells, with the small number of undifferentiated cells, histiocytes, and other primitive mononuclears commonly seen in association with neovascular tissue.

In cases in which the process appeared to have broken through into the

vitreous, retinal folds, maceration, and other vitiating factors were usually present, and it was felt the appearance was almost certainly that of an artefact. For purposes of comparing the characteristics of cases showing unusual quantities of vaso-formative tissue with the average characteristics of the total series of 100 cases, the abnormal cases were divided into three groups. Vaso-formative tissue was commonly more abundant at the anterior limit of retinal vascularization in the region where maximal vascular growth occurs, a high proportion of cases showing a growing tip. In this area, clumps of mesenchymal and endothelial cells of moderate dimensions were classified as within normal limits (Fig. 2 *a*).

GROUP A.—These were defined as showing clumps of vascular and vaso-formative tissue of moderately increased dimensions at the growing tip. Three cases fell into this category (Fig. 2 *b*).

GROUP B.—These were of intermediate significance; they showed larger single masses anteriorly or minor masses recurring posteriorly. Three cases fell into this group (Fig. 2 *c*).

GROUP C.—These showed larger masses both at the anterior limit of vascularization and recurrently behind this area (Fig. 2 *d*).

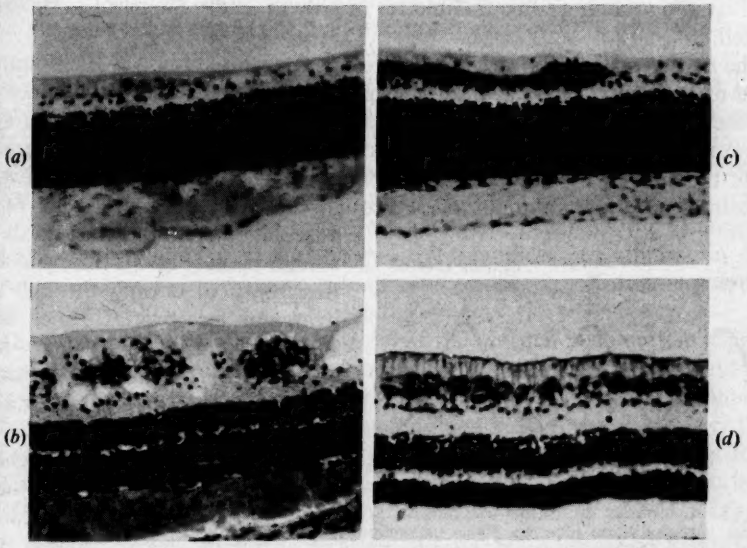

FIG. 2. (*a*).—Usual quantity of vaso-formative tissue at growing tip of retinal vessels.
(*b*).—Group "A" type of vaso-formative tissue proliferation. ×120.
(*c*).—Group "B" type of vaso-formative tissue proliferation. ×120.
(*d*).—Group "C" type of vaso-formative tissue proliferation. ×120.

Retinal vascular development is usually described as commencing at the 100 mm. (approx. 4 months) stage in the foetus, as endothelial budding

arising from the hyaloid vessels (Versari, 1910; Bach and Seefelder, 1914; Mann, 1928). This process of advancing syncytial columns of mesenchymal cells followed by canalization spreads peripherally in the nerve fibre layer and also towards the inner nuclear layer, and is said to be complete by the eighth month of intra-uterine life.

Michaelson (1948), in a detailed study of six human foetuses injected with Indian ink, showed that vascularization spreads from the optic disc as four complexes destined to become the definitive nasal and temporal vessels; the vascular complexes, roughly diamond-shaped, are at first separated by avascular retina; at an early stage, the advancing columns of mesenchymal cells present a continuous front with probably a forward peak at the centre of each complex until vascularization is complete. Michaelson also showed that growth took place chiefly from the veins, and he stressed the difference between the small mesh of the developing capillary net with its large-bore, jointed, angular vessels, and the small-bore of the adult net with larger, rounded vessels. He showed that the peri-arterial capillary free zone is more marked in the developing vascular system, and he found no difference in growth-rate between the nasal and temporal vessels, showing that their simultaneous arrival at the periphery was due to the temporal vessels budding out first and attaining some millimetres of growth before the nasal complexes started.

The first step to assess vascular development in this study was the examination of horizontal sections and the estimation of the distance in millimetres of vascular or vaso-formative tissue from the ora: one slide from each eye was examined and the most advanced point noted. Theoretically, inaccuracy might be due first to the size of the capillary net; secondly to the fact that a horizontal section might not pass through the most advanced peak of a vascular system advancing in a similar plane (Fig. 3); thirdly to the possibility of cutting through an avascular periarterial zone, and missing the growing capillary net in front of it (because of displacement of tissue by maceration and technical procedures).

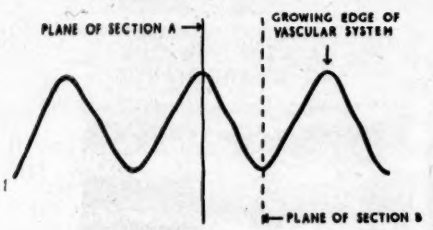

FIG. 3.—Inaccurate estimation of progress of retinal vascularization due to plane of section (arrow) coinciding with plane of growth.

Duration of pregnancy is generally considered to be the most accurate guide to the prematurity of an infant, but in view of the relationship between birth weight and retrolental fibroplasia, the results were first related to weight, and it was found that in the under-2 lb. group of infants (24–32 weeks' pregnancy) retinal vascularization had never reached the ora. In babies of heavier weights there might be peripheral areas of avascularity; these were present to a very minor degree up to 5 lb. (the heaviest infant in this series).

In view of the variation of the rate of retinal vascularization shown in the results of examination of the whole group of 100 premature infants (in the under 2 lb. group, almost 5 mm. distance), a series comprising foetuses obtained at hysterotomy and stillborn infants was first examined to eliminate the error due to the effects of extra-uterine growth. This showed that:

(*i*) the retina was avascular at from 8 to 12 weeks;
(*ii*) early vascularization was present at the posterior pole at 12 to 13 weeks;
(*iii*) vessels and vaso-formative tissue, which had reached the equator by 19 to 28 weeks, were 5/8 of the way to the ora by 25 to 35 weeks, and 6/8 of the way by 29 to 32 weeks, and had reached the ora by 29 weeks.

Next, in order to minimize the error due to the plane of the section not passing through the most advanced point of the growing tip and to give some idea of the width of this actively proliferating area at the ages and weights examined, coronal sections were cut (Fig. 1). The results showed a variation in the age at which the vessels reached the ora of from 29 to 35 weeks (though all cases were in the 2 to 3 lb. group). In the youngest case examined (a 28 weeks' pregnancy, 1 to 2 lb. group), the retinal vessels had grown 5/8 of the distance to the ora. Active vaso-formative tissue was present anteriorly in seven cases and was distributed over an area of approximately one-quarter of the total distance from the optic disc to the ora (9 mm.). In all these cases, periodic acid-Schiff (PAS) positive granules were present in the proliferating area.

To assess and compare the effects of post-natal vascular growth, a comparable series of nine live births ranging in age from 2 to 12 days were similarly examined by vertical section; they showed the same variation in growth rate, though the ora appeared to be vascularized slightly later, at from 32 to 36 weeks. Active vaso-formative tissue was present to a minor degree in only four cases, and the area in which it occurred was usually less than in the stillbirth series. PAS positive granules were present in only three cases, being absent in two cases showing active vaso-formative tissue—one of 2 days old and one of 7 days old.

Other interesting points noted were that the unusual proliferative changes found in Case 39 were shown to be extensive in another dimension, and that one other stillbirth was shown to possess vaso-formative tissue in unusual quantity when examined by coronal section and would have met the criteria set for Group B cases in the horizontal section examination, thus underlining the patchy distribution of these proliferation changes (Fig. 4*a* and *b*, overleaf).

If conclusions may be drawn from such a small number of cases, the suggestion is that vascular growth and particularly any unusual degree of proliferation is slowed down after birth.

The examination of the eyes injected with Indian ink added nothing to our knowledge of the times and weights at which vessels reached the ora, confirming only the variability already established. The flat preparations showed that in a one-week-old infant of 10 lb. birth weight, the foetal type of capillary net had practically disappeared. In a one-week-old full-term

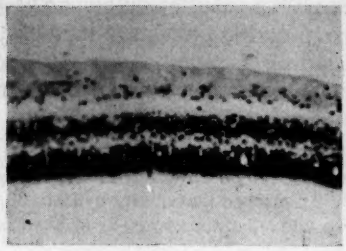

FIG. 4 (*a*)

FIG. 4 (*a*).—Case 52 (R) horizontal section showing normal quantities of vaso-formative tissue. ×120.

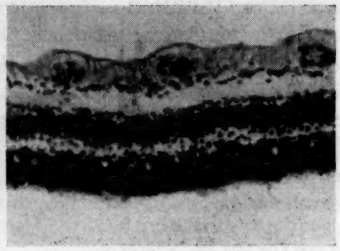

FIG. 4 (*b*)

(*b*).—Case 52 (R) coronal section through same area showing Group "B" type of vaso-formative tissue proliferation. ×120.

infant of 6 lb. birth weight, it was patchily present at the periphery. At 5 lb. 5 oz. more was present, and at lower weights the net predominated peripherally. These preparations also showed the difference between the peripheral arterio-venous arcades of the completed vascular system and the open actively branching terminations of the developing system (Fig. 5). Some cases showed a double peripheral net, another explanation of the appearance of increased vascularity noted in the premature eye.

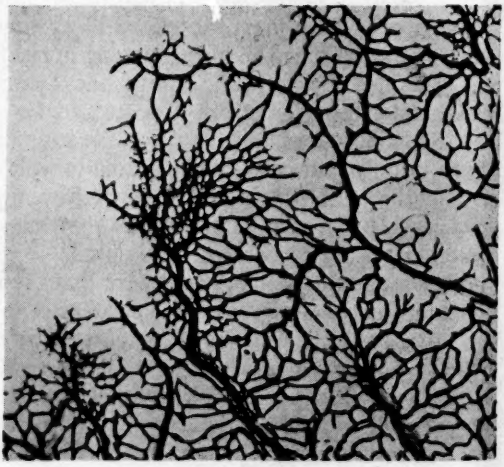

FIG. 5.—Injected retina of full-term infant (birth weight 4½ lb.) to show open, actively-branching, embryonic type of capillary net at periphery and adult vascular pattern centrally. ×29.

Michaelson's observations on developing and mature vascular systems were thus confirmed, and the possibility of visualizing the developing circulation in plan gave considerable aid in the interpretation of histological pictures seen on section. For instance, Fig. 6 shows the irregularity of vascular growth, with dense developing capillary systems near veins and relatively avascular areas near arteries. A section through this proliferating area, such as (1) and (2), could easily show the columns and minor masses of endothelial

cells such as are seen at the advancing vascular edge. A section through (3), where the endothelial cells begin to branch out fanwise soon after leaving the vein, might give a whorl-like appearance.

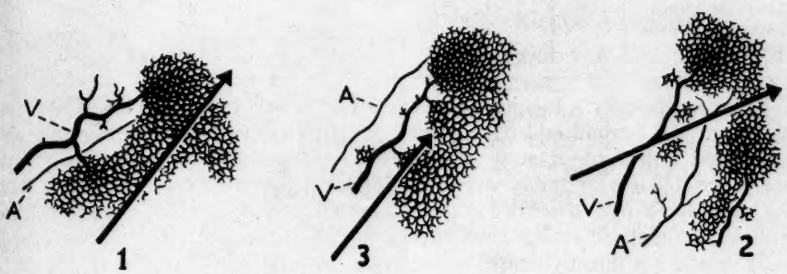

FIG. 6.—Irregularity of vascular growth with dense proliferating capillary systems near veins and relatively avascular areas round arteries. Section through 1 could show a column, and through 2 a minor mass of vaso-formative tissue, and through 3 a whorl-like appearance.

The more marked proliferations seen in the cases of Group C are less easily accepted as a normally developing vascular system, but might be regarded as an upper limit of normal variations in the course of development.

To determine whether the vascular changes described in Groups A, B, and C were within normal limits or had any pathological significance in relation to retrolental fibroplasia, the findings in these groups were analyzed and compared with the rest of the 100 premature births which showed no such changes. The results were as follows:

Weight Incidence

Birth Weight (lb.)	No. of Cases	Percentage of Cases	Incidence of this birth weight for comparison in the analysis of 100 cases (per cent.)
4–5	1	8	4
2–3	9	69	55
Under 2	3	23	24

This analysis shows a preponderance in the under-3 lb. group, but, whilst a relationship with low birth weight is similar to the incidence of retrolental fibroplasia, this characteristic may be attributable to the dominance of this birth weight group in the total series, and is, therefore, of doubtful significance.

Eye Size.—No special relationship was established between the vascular changes and eye size. Five cases were below and eight cases were above the average equatorial diameter for their weight group.

Extra-uterine Age.—The age relationship of the cases in Groups A, B, and C follows the general trend of distribution in the 100 cases examined. It should be noted that one case was stillborn, one lived 25 minutes, and the oldest lived for 2 months. The duration of pregnancy varied from 29 to 37 weeks.

Age	No. of Cases	Percentage of Cases	Incidence of age groups in the total series of 100 cases for comparison (per cent.)
Stillbirths	1	8	13
Less than 1 day	3	24	26
Less than 1 week	7	52	47
More than 1 week	1	8	5
Unknown	1	8	5

Vascular Maturity.—In only three cases had vessels actually reached the ora, and even in a 2-month-old baby patchy peripheral avascular areas were present. Compared with the average development figures, in five cases the retinal vessels had penetrated less far and in seven cases further towards the ora. This shows that the vascular changes described in the cases in Groups A, B, and C were associated with an incompleted retinal circulation and that the degree of incompleteness is variable and not directly related to the degree of angioblastic activity.

Retinal Folds.—These were present in twelve cases (92 per cent.). This might be indicative of the degree of *post-mortem* distortion, but in view of the incidence of folds in 90 per cent. of the total series of 100 cases, this result is unhelpful.

Retinal Congestion.—This was a prominent feature in ten cases (77 per cent.) compared with 20 per cent in the total series.

Retinal Haemorrhages.—These were present in five cases (39 per cent.), the incidence in the total series being also 39 per cent. The haemorrhages were of slight degree only, except in two cases, in which they were being invaded by endothelial cells.

Uveal Tract Congestion.—This was present in twelve cases (92 per cent.), an increase over the incidence in the total series of 57 per cent.

Inflammatory Infiltration.—This occurred in five cases (39 per cent.), comparable with the incidence in the total series of 40 per cent. In only one case was it at all extensive, when it was considered to be a form of extra-medullary haemopoiesis and then bore no apparent relationship to the retinal changes.

Cause of Death.—Anoxia usually played a part, but the stated causes were probably often unreliable, as *post-mortem* examinations were not always possible.

Associated Congenital Abnormality.—This was present in only one case of Fallot's tetralogy with absence of the right kidney and ureter and laryngeal stenosis.

Position of Vascular Changes.—This was most commonly from the equator forward to the anterior limit of vascularization, *i.e.* in the area of most active growth.

In considering these results it should be remembered that, for obvious reasons, the material included few full-term infants or babies who had lived for many weeks.

To sum up, the thirteen cases which fell in Groups A, B, and C showed an unusually marked degree of angioblastic activity, most commonly in front of the equator (in the area of most active vascular growth). These cases came predominantly from the 2 to 3 lb. birth weight group. Gestation times were 28 to 37 weeks and extra-u erine ages varied from stillbirth to 2 weeks. The affected retinae were usually not fully vascularized and only two cases showed haemorrhages of any size. The incidence of retinal and

choroidal congestion was found to be greater than in the total series, but it will be seen that no causal association was, in fact, established, and that the incidence in the lower weight groups, a characteristic of debatable significance, and prematurity are the only doubtful links with retrolental fibroplasia.

FIG. 7.—Separation of clear from pigmented cells of the pars plana of ciliary body. ×120.

Retinal Coaptation

Attention was directed particularly towards any association of separation of the clear and pigmented cells of the pars plana (Fig. 7), or separation of the oral and adjacent retina from the underlying pigment epithelium, and of retinal and oral folds with any uveal or retinal characteristic which might be associated with retrolental fibroplasia. The results were as follows:

(1) *Separation of the clear from the pigmented cells of the pars plana.*—This occurred in varying degrees in eight cases:

Birth Weight	Under 5 lb.	Under 4 lb.	Under 3 lb.	Under 2 lb.	Unknown	Total
No. of Cases	0	0	5	2	1	8

(2) *Separation of the clear and the pigmented cells from the basement membrane*

(*a*) *Moderate* (34 cases):

Birthweight	Under 5 lb.	Under 4 lb.	Under 3 lb.	Under 2 lb.	Unknown	Total
No. of Cases	1	3	22	7	1	34

(*b*) *Extensive* (3 cases):

Birthweight	under 3 lb.	Under 2 lb.	Total
No. of Cases	2	1	3

The incidence of infiltration (42 per cent.) and of congestion (60 per cent.), of the uveal tract are comparable to an incidence in the total series of 100 cases of 40 and 57 per cent. respectively. The corresponding figures for separation of the clear cells of the pars plana only were 62 per cent. and 50 per cent.—no significant difference for such a small number of cases.

(3) *Oral Retinal Folds.*—These were present in 95 per cent. The earliest sign of this oral folding was a separation of the oral retina and neighbouring clear cells of the pars plana from the subjacent pigment epithelium, which showed a notch directed forwards and inwards. Later the pigment epithelium separated from the basement membrane and became plicated, leading to a separation of the clear and pigmented cells for variable distance along the pars plana, reaching in some cases to the ciliary processes (Fig. 8*a*, *b*, *c*, and *d*). In one case, separation of the oral retina and clear cells of the pars plana was observed with no oral fold.

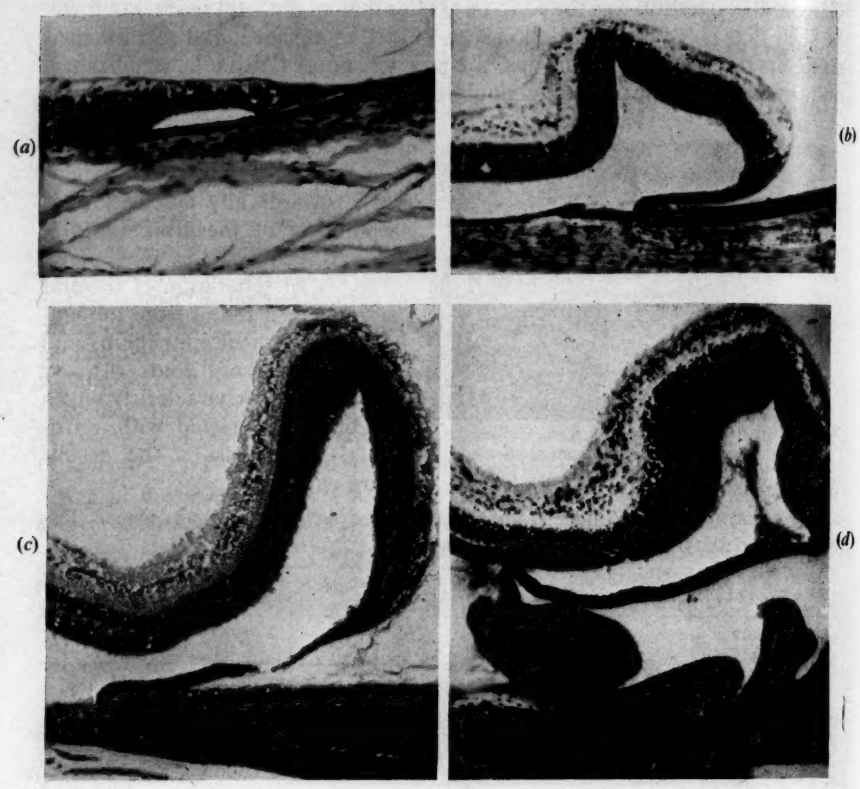

FIG. 8 (*a–d*).—Four stages in formation of retinal oral fold. ×120.

With a view to interpreting these results, consideration was given to the development of coaptation of the inner and outer layers of the optic vesicle.

At the 21-mm. stage of development (approx. 7 weeks' gestation), filaments from the external limiting membrane of the inner layer of the optic cup, representing the fore-runners of the rods and cones, come into contact with the pigmented epithelium of the outer wall. Coaptation of the inner and outer layers occurs at a later date, but clearly there can be no real cohesion between the layers until growth is complete. In the ciliary region the adhesion between the clear and pigmented epithelium is said to be firm (Mann, 1928). This ciliary region is formed by the growth of the margin of the optic cup past the equator of the lens, starting at the 48-mm. stage (approx. 10 weeks' gestation). At first, the layers of the cup are widely separated by the cavity of the primary optic vesicle (Fig. 9). At about the 65-mm. stage (approximately 12 weeks' gestation), adhesion is beginning between the inner and outer layers except just at the growing tip. The development of the ciliary region is a slow process: the folded growing edge extends, leaving the pars plana behind. At 5 months this structure is hardly visible; by 7 months the ora

FIG. 9.—48-mm. stage embryo (about 11 weeks' gestation) showing separation of inner and outer layers of optic cup and growth of margin of cup past equator of lens.

has reached the middle of the ciliary muscle, by 8 months the posterior third, and by 9 months (Fig. 10) the posterior border. (Bach and Seefelder, 1914; Mann, 1928).

To sum up these observations on retinal coaptation, developmental considerations preclude the anticipation of any firm adhesion between the layers of the optic cup in a premature eye. That cohesion is in fact poor is borne out by the frequency of retinal folding (90 per cent.), separation of the clear cells of the pars plana (8 per cent.), and separation of the clear and the pigmented cells (37 per cent.), reported in this series. If these folds and separations are artefacts, which is probable, they demonstrate that little cohesion existed. The late development of the pars plana, first visible at the fifth month, makes it unreasonable to hold responsible any noxa acting before that date for detachment of the retina in this area, and, furthermore, no evidence of significant inflammation or other cause was found

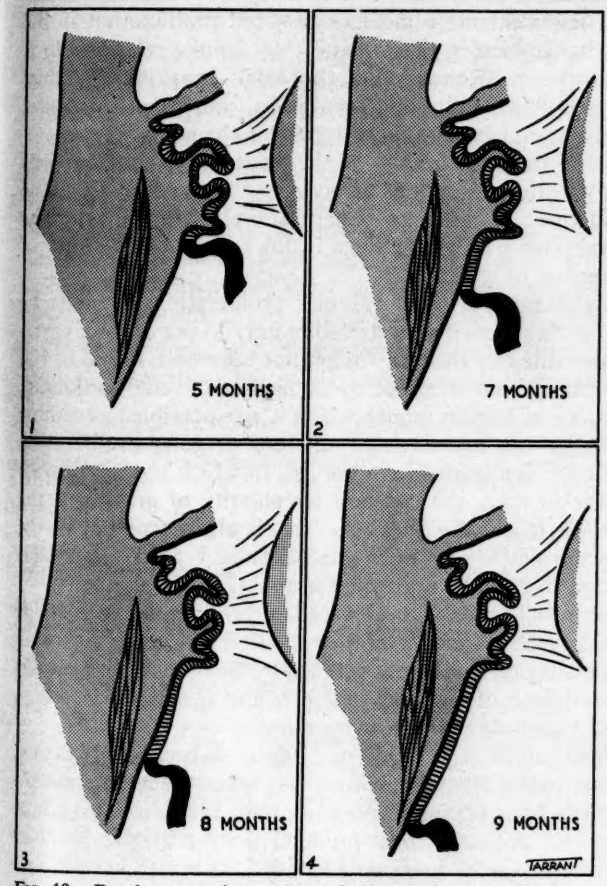

FIG. 10.—Development of pars plana of ciliary body and oral retina.

in this series. The significance of the true detachment of the clear cells of the pars plana would, therefore, seem to be that of a detachment developing in an immature eye, whatever the cause. Such detachments (due to many causes other than retrolental fibroplasia, *i.e.* trauma, infection, etc.) may in fact be seen in infant eyes.

Discussion

This investigation shows that in the retinae of 13 per cent. of premature babies there are signs of an unusual degree of angioblastic activity. This change is commonest in the lower birth weight groups and is associated with incomplete retinal vascularization. The literature on the pathology of retrolental fibroplasia has been reviewed by Ashton (1954), and there seems general agreement that thickening of the nerve fibre layer of the retina due to endothelial and to a lesser extent to mesenchymal cell proliferation is the earliest microscopic change seen in the disease. No definite relationship is established to haemorrhage. Retinal and choroidal congestion, retinal oedema, defects in the internal limiting membrane, and other structural abnormalities may or may not be due to *post-mortem* change.

Any consideration of the relationship of the endothelial proliferations discussed in this study to those described as occurring in the early stages of retrolental fibroplasia must of necessity remain entirely speculative. The histological picture represents only one stage in the continuous process of progression and regression of growth.

In assessing the significance of these vascular proliferations, it must be remembered that whilst they were demonstrated in only 13 per cent. of cases, it is not possible to say definitely that they might not have been found in 100 per cent. had the specimens been examined by the method of serial sectioning. The few flat preparations of human retinae which it was possible to examine after intravascular Indian ink injection showed very irregular proliferation both at the growing edge and behind. In the cat, in which the retinal vascular system is similar to man, the constant irregularity of growth of the developing blood vessels is a striking feature. This is also borne out by the fact that vertical sections of one human case showed typical glomerular whorls in an area judged normal by horizontal section (Fig. 4 *a* and *b*).

Amongst our material all gradations of vascular proliferation are to be seen, from the normal to the extreme, and their division into categories is an arbitrary process depending on the criteria adopted by the individual observer. Broadly speaking, the differences are ones of degree and situation, *i.e.* arising from more developed vessels or at the growing margin.

Whilst some of these proliferations may be destined to become abnormal, it is impossible to say, unless vitreous invasion has taken place, that pathological vascular growth has occurred, and even then regression may take place later. That some degree of over-production of primitive vascular tissue forms a common feature of developing circulations is well known, and may well be related to variations in oxygen tension within the foetal tissues.

Keibel and Mall (1910-12), discussing normal vascular development, stated that a capillary net always exists at the periphery of the growing vascular tree. The main vessels arise by utilization of a single channel in the mesh and the co-incidental atrophy of the remainder.

These vascular endothelial changes have been considered to form the nucleus of retrolental fibroplasia (Reese and others, 1952), but, since they may so easily be explained by considerations of development and its anomalies, it is perhaps wiser to label them as an imponderable characteristic of the developing retina.

Summary

(1) The eyes from one hundred premature infants were examined with particular reference to the processes of retinal vascularization and coaptation.

(2) 13 per cent. of these cases showed an unusual degree of angioblastic activity, indicated by increased quantities of vaso-formative tissue in the nerve fibre layer. This change was associated with no characteristic of retrolental fibroplasia apart from low birth weight. There was no evidence to suggest that it was beyond the upper limits of normality.

(3) Separation of the clear from the pigmented cells of the pars plana, and of the clear and pigmented cells from the basement membrane, and the frequent, retinal folds were thought to be artefacts attributable to differential shrinkage of the coats of the eye, indicating only that adhesion between the layers of the optic cup is poorly developed in the premature eye.

(4) No evidence of a nucleus of retrolental fibroplasia was found, apart from the incomplete retinal vasculature, which is characteristic of prematurity.

I wish to thank Dr. Norman Ashton for his encouragement and advice. My thanks are also due to the medical illustration department of the Institute of Ophthalmology for photographs and diagrams. I am particularly grateful to Messrs. G. E. Knight, A. McNeil, and A. Johnson for technical assistance, and to Miss E. FitzGerald for secretarial help.

REFERENCES

ASHTON, N. (1954). *British Journal of Ophthalmology*, **38**, 385.
BACH, L., and SEEFELDER, R. (1914). "Atlas zur Entwicklungs-geschichte des menschlichen Auges". Engelmann, Leipzig and Berlin.
DIXON, J. M., and PAUL, E. V. (1951). *Amer. J. Ophthal.*, **34**, No. 5, (May) Pt. 2, p. 182.
FRIEDENWALD, J. S., OWENS, W. C., and OWENS, E. U. (1952). *Trans. Amer. ophthal. Soc.*, **49**, 207.
HEATH, P. (1950). *Arch. Ophthal. (Chicago)*, **44**, 245.
——— (1951). *Amer. J. Ophthal.*, **34**, 1249.
KEIBEL, F., and MALL, F. P. (1910–12). "Manual of Human Embryology". Lippincott, Philadelphia and London.
MANN, I. (1928). "The Development of the Human Eye". University Press, Cambridge.
MICHAELSON, I. C. (1948). *Trans. ophthal. Soc. U.K.*, **68**, 137.
REESE, A. B., and BLODI, F. C. (1951). *Amer. J. Ophthal.*, **34**, 1.
——— ———, and Locke, J. C. (1952). *Ibid.*, **35**, 1407.
SERPELL, G. (1954). *British Journal of Ophthalmology*, **38**,
TERRY, T. L. (1942). *Amer. J. Ophthal.*, **25**, 203.
VERSARI, R. (1910). *Anat. Anz.*, **35**, 105.
WOLFF, E. (1950). *Proc. roy. Soc. Med.*, **43**, 227.

Brit. J. Ophthal. (1954) **38**, 460.

POLYSACCHARIDE GRANULES IN ASSOCIATION WITH DEVELOPING RETINAL VESSELS AND WITH RETROLENTAL FIBROPLASIA

BY

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THE occurrence of polysaccharide in the retina has been known for some time, but there has been no uniformity of opinion as to its exact situation and functional significance. Glycogen has been described in the retina of the frog (Ehrlich, 1883; Brammertz, 1915; Schmitz-Moorman, 1927), the rabbit (Brammertz, 1915), the cat, and man (Kojima and Nagao, 1953), and in the rabbit retina after subcutaneous and intravitreal injection of dextrose (Nakashima, 1926). On the other hand, Best (1903) was unable to demonstrate this substance in the frog retina.

Until the introduction of the periodic acid-Schiff method, first used by McManus (1946, 1948) to demonstrate mucin histologically and subsequently elaborated into a histochemical technique for polysaccharide by Hotchkiss (1948), staining methods for identifying polysaccharides in tissues were empiric (Carpenter and others, 1951), as in the use of iodine (Bernard; see Creighton, 1896) and carmine (Best, 1906). With the application of the PAS method to the adult human retina, the internal limiting membrane, the peripheral portions of the rods and cones, and the basement membrane of the vascular tree were found to stain positively (Friedenwald, 1949; Day, 1950). In addition, intensely staining globules in the nerve fibre layer of degenerating retinae have been described by Day (1950).

In infants up to 2 years of age, Friedenwald (1949) showed that the retinal vascular basement membrane is much thinner and less easily seen than in adults; in the premature human infant, he found this basement membrane visible in the larger vessels only and not at all in the capillaries. Friedenwald concluded that it was not possible to say whether the membrane was non-existent in foetal capillaries or merely so thin and delicate as to be invisible.

In the developing rabbit retina, Bembridge and Pirie (1951) described granules staining positively with PAS, which were found mainly in the inner retinal layers in the young rabbit between the eighth day and the end of the third week of life, but no relation to developing retinal vessels was reported. Compared with the human eye, the rabbit eye at birth is premature, in that the retina is not fully developed, the hyaloid vascular system still persists, and the lids are closed.

Recently, Kojima and Nagao (1953) have reported similar granules staining with PAS in the retinae of man and the cat, and Ashton (1954) has noted the occurrence of large numbers of PAS-positive granules in the retina in early retrolental fibroplasia.

This latter observation stimulated the investigations recorded in this paper of the occurrence of polysaccharide in the developing human retina. These investigations were later extended to the kitten retina, in association with studies on the aetiology of retrolental fibroplasia (Ashton and others, 1953, 1954), and also to the retina of the young rabbit.

It was found that PAS-positive granules occur normally in association with proliferating mesenchymal cells in the developing retinal vasculature in man, the rabbit, and the cat, and pathologically in the retina in the early stage of retrolental fibroplasia in man and the kitten. The granules were found to consist of glycogen and their histochemical identification is described.

Material

(1) *Early Retrolental Fibroplasia.*—Thirteen eyes of seven cases of early retrolental fibroplasia.

(2) *Live-born Premature Infants.*—46 eyes of 27 infants of birth weight 1 lb. 3 oz. to 5 lb. 15 oz. and gestation period 26–36 weeks. Death was due to prematurity *per se* or associated pulmonary complications with an asphyxial mode of death in most cases.

(3) *Live-born Mature Full-term Infants.*—Four eyes from two infants were examined. Death was due to congenital heart disease in one instance, and to hydrocephalus in the other.

(4) *Stillborn Premature Infants.*—25 eyes of fourteen infants, one an anencephalic, were examined. The period of gestation ranged from 25 to 39 weeks.

(5) *Stillborn Mature Full-term Infants.*—Three eyes from two infants.

(6) *Foetuses.*—Seven were examined, one of 8 weeks' gestation period, three of 12 weeks', and one each of 16, 18, and 19 weeks' gestation.

(7) *Rabbit.*—A 14-day-old rabbit was examined, one eye being fixed in 10 per cent. formol saline and the other in Bouin's fluid.

(8) *Kittens.*—The retinae of several of the kittens used in experiments on the production of retrolental fibroplasia, described by Ashton and others (1954), were examined. Both normal kittens and those with new-vessel formation, induced by treatment with oxygen, were examined.

Methods

The specimens were all fixed in 10 per cent. formol saline, embedded in celloidin, and sectioned. Each specimen was stained with haematoxylin and eosin and by the periodic acid-Schiff method (PAS). In determining the nature of the granules, various polysaccharide stains and enzyme digestion studies were used:

(1) *Differential Staining:*

(*i*) PAS
(*ii*) Iodine
(*iii*) Toluidine blue
(*iv*) Best's carmine
(*v*) Sudan black
(*vi*) Southgate muci-carmine
(*vii*) Schiff's reagent without previous treatment of the section with periodic acid.

To demonstrate the activity of the particular preparation of stain, a section rich in the substance for which the stain is specific was stained and examined, *e.g.* rabbit's liver (glycogen) was used for iodine and Best's carmine, cornea (mucopolysaccharide) for toluidine blue metachromasia, and conjunctival mucous secreting cells (mucin) for Southgate muci-carmine.

(2) *Enzyme Studies*

(*i*) Hyaluronidase.—The preparation used was "Rondase" (Evans), a form of mammalian testis hyaluronidase, and this was used with acetate buffer as described by Pearse (1953).

(*ii*) Diastase.—Malt diastase (1 per cent.) in saline was used, a buffer not being required (Pearse, 1953).

In each case an active specimen and a boiled control were used, and both sections were subsequently stained with PAS.

A standard specimen of early retrolental fibroplasia (R.L.F.30 L.) was used for most of the experiments because of the much greater numbers of polysaccharide granules present. Identical tests were later applied to the retinae of normal human infants, the rabbit, and the kitten, thereby demonstrating the identity of the granules in these several species.

Observations

Granules of polysaccharide staining positively with PAS in the immature retina fixed in formol saline, are of varying size and purplish red in colour. They occur both intra- and extra-cellularly and are referred to below as *PAS-positive granules*. As far as possible, the distribution of the granules in the retinae is described according to the scheme illustrated in Fig. 1.

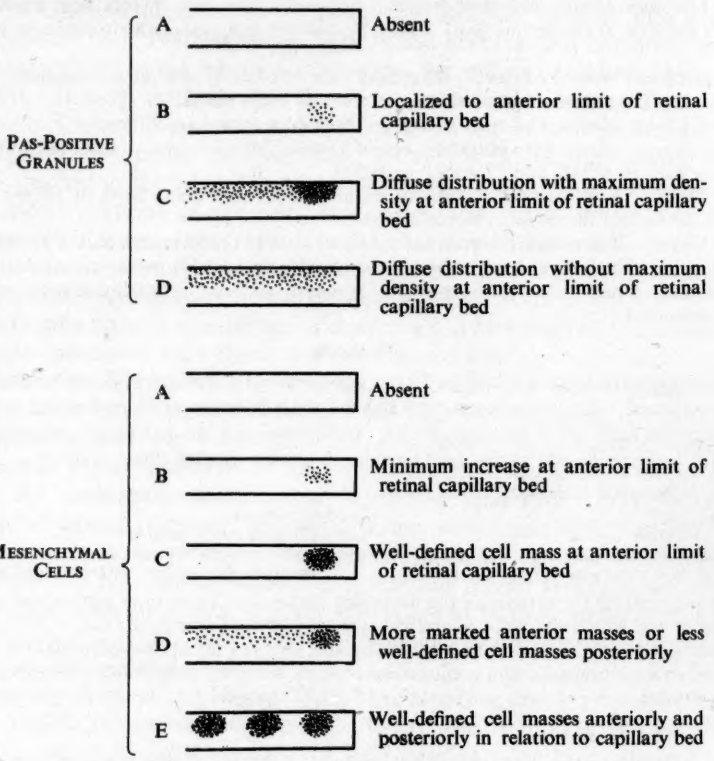
PAS-Positive Granules
A
Absent
B
Localized to anterior limit of retinal capillary bed
C
Diffuse distribution with maximum density at anterior limit of retinal capillary bed
D
Diffuse distribution without maximum density at anterior limit of retinal capillary bed
Mesenchymal Cells
A
Absent
B
Minimum increase at anterior limit of retinal capillary bed
C
Well-defined cell mass at anterior limit of retinal capillary bed
D
More marked anterior masses or less well-defined cell masses posteriorly
E
Well-defined cell masses anteriorly and posteriorly in relation to capillary bed

FIG. 1.—Classification of PAS-positive granule distribution and mesenchymal cell distribution.

The nomenclature of the cells of mesodermal origin, constituting and associated with the developing retinal vascular tree, is confused. In previous accounts of the early histopathology of retrolental fibroplasia, the term " increased glial and endothelial cells " has been used. It appears that the cells with dark-staining nuclei of spindle shape, which were regarded as " glial cells ", are in fact cells of mesenchymal origin (Ashton, 1954). Larger cells with larger pale-staining nuclei are endothelial cells, and these are more intimately associated with canalized capillaries.

We have, therefore, described the actively proliferating cells (" glial cells ") as *mesenchymal cells*, and it will be shown that it is these particular cells which are associated with the presence of polysaccharide granules. As development of the retinal vessels proceeds in three planes, mesenchymal cell masses may occur anywhere in relation to the vascular tree; the distribution of cells is therefore described according to the scheme illustrated in Fig. 1.

Occurrence of Polysaccharide Granules

(1) *Early Retrolental Fibroplasia.*—Large numbers of PAS-positive granules were found in the inner retinal layers in intimate association with the areas of mesenchymal cell proliferation, as described by Ashton (1954) in the early stage of retrolental fibroplasia (Figs 2 and 3). The granules were found to be strictly intra-retinal in distribution, and where capillary buds grow out from the retina into the vitreous, whether canalized or not, granules were found only in the adjacent retina and not in association with the intravitreal out growths. Furthermore, the density of

FIG. 2.—Early retrolental fibroplasia. High-power view of intra-retinal mesenchymal cell masses (RLF 30 L). Haematoxylin and eosin. ×500.

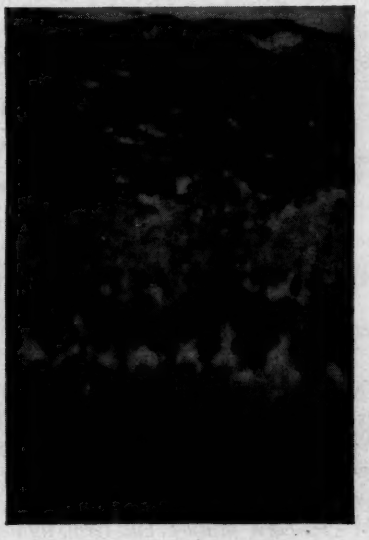

FIG. 3.—Early retrolental fibroplasia. High-power view showing relation of PAS-positive granules to mesenchymal cells in retina. (Cf. Fig. 2). PAS and haematoxylin. ×500.

granules was greater in relation to proliferating cell masses in the retina than to recently canalized newly formed vessels.

(2) *Live-born Premature Infants.*—The results are summarized in Tables I and II (see also Fig. 1). Of the 46 live-born premature infants' eyes, 76 per cent. showed an increase of mesenchymal cells, of varying extent in relation to the developing retinal vessels, and in 50 per cent. the cells were present as well defined masses (Fig. 4, opposite). Three retinae showed mesenchymal cells of Group E distribution; these are of particular interest in that the retinae are on the borderline between those with normally developing vessels and those showing the earliest changes of retrolental fibroplasia (Ashton, 1954; Ward, 1954).

TABLE I

INCIDENCE OF MESENCHYMAL CELLS AND PAS-POSITIVE GRANULES IN RETINAE OF LIVE-BORN PREMATURE INFANTS

Distribution of Mesenchymal Cells	Distribution of PAS-Positive Granules				Total
	A	B	C	D	
A	6	2	1	2	11
B	1	3	4	4	12
C	1	1	6	2	10
D	0	4	5	1	10
E	0	0	3	0	3
Total	8	10	19	9	46

TABLE II

INCIDENCE OF MESENCHYMAL CELLS AND PAS-POSITIVE GRANULES IN RETINAE OF LIVE-BORN PREMATURE INFANTS (PERCENTAGE)

Formations	Type of Distribution	per cent.
Mesenchymal Cells (76 per cent.)	(*a*) well-defined masses	50
	(*b*) minimal increase	26
PAS-Positive Granules (83 per cent.)	(*a*) localized to anterior limit of capillary bed	22
	(*b*) diffuse with maximum density at anterior limit of capillary bed	41
	(*c*) diffuse distribution	20

PAS-positive granules were found in 83 per cent. of the live-born premature infants' eyes, Group C distribution being the commonest finding (Fig. 5, opposite).

Whatever the distribution of these granules, in no case were they found in front of the most anterior limit of the capillary bed, and when present, they were always found in the inner retinal layers, which are nourished by retinal capillaries.

(3) *Live-born Mature Full-term Infants.*—None of the four retinae examined (two cases) showed an increase in mesenchymal cells, and there was a complete absence of PAS-positive granules from the inner retinal layers.

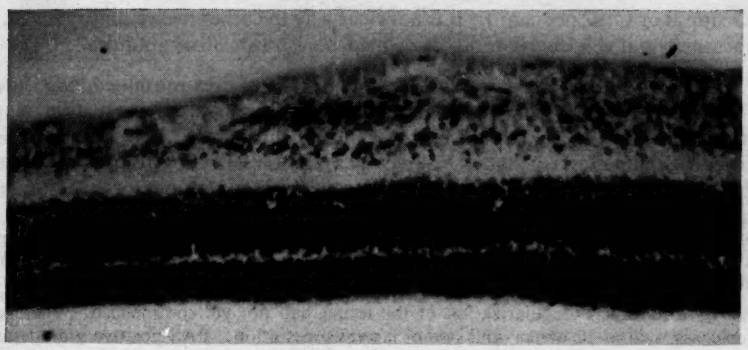

FIG. 4.—Retina of live-born premature infant (birth weight 2 lb. 6 oz., 32 weeks' gestation), showing mass of mesenchymal cells at anterior limit of retinal vascular tree. Patent capillaries situated to right of cell mass, *i.e.* towards optic disc. (PM 110 L). Haematoxylin and eosin. ×120.

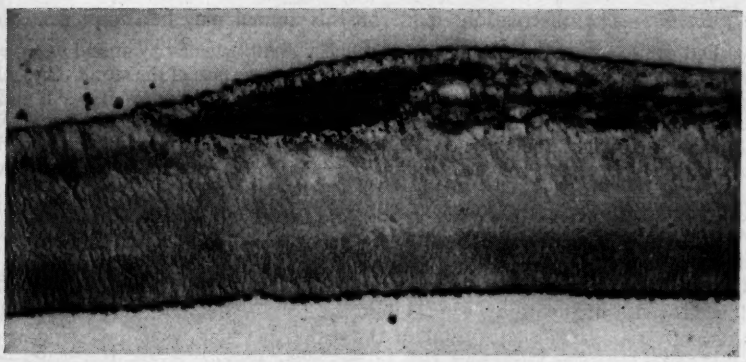

FIG. 5.—Retina of live-born premature infant (birth weight 2 lb. 6 oz., 32 weeks' gestation), showing relation of PAS-positive granules to retinal capillaries. The greatest density of granules is in relation to the mesenchymal cell mass. (Cf. Fig. 4). (PM 110 L). PAS. ×120.

(4) *Stillborn Premature Infants.*—The results are summarized in Table III, which shows that 23 of the 25 eyes examined showed PAS-positive granule distribution

TABLE III

INCIDENCE OF MESENCHYMAL CELLS AND PAS-POSITIVE GRANULES IN RETINAE OF STILLBORN PREMATURE INFANTS

Distribution of Mesenchymal Cells	Distribution of PAS-Positive Granules				Total
	A	B	C	D	
A	0	0	0	0	0
B	2	3	7	0	12
C	0	5	7	0	12
D	0	1	0	0	1
E	0	0	0	0	0
Total	2	9	14	0	25

of Group B or C (92 per cent.). It is not clear why the other two retinae, with Group B mesenchymal cell distribution, contained no PAS-positive granules.

(5) *Stillborn Mature Full-term Infants.*—Of the three eyes examined, one showed no PAS-positive granules, although mesenchymal cells were present in masses (Group E), but poor fixation possibly explains this result. The other two eyes showed a minimal mesenchymal cell increase (Group B) with Group B distribution of PAS-positive granules. This is of interest since the retinae of live-born full-term infants showed no PAS-positive granules.

(6) *Foetuses.*—The results of examining the retinae of seven foetal eyes may be summarized thus:

(*a*) Eyes in which vascularization of the retina had not yet commenced. There were three cases, two of 12 weeks' and one of 8 weeks' gestation. PAS-positive granules were not present in any of these retinae.

(*b*) Eyes in which vascularization of the retina had commenced. At the 12, 16, 18, and 19 weeks stages, all showed PAS-positive granules in relation to the developing retinal vessels the maximal density of granules being seen anteriorly.

(7) *Rabbit.*—The observations made on this animal may be summarized thus:

(*a*) Left eye fixed in 10 per cent. formol saline. With haematoxylin and eosin, well-developed retinal vessels were found, mainly on the inner surface of the retina. The rabbit has a membrana vasculosa retinae type of retinal circulation (Wolff, 1948). PAS-positive granules were present in the inner retinal layers in relation to the blood vessels.

(*b*) Right eye fixed in Bouin's fluid. No vessels were present in this specimen apart from a capillary at the equator on one side, and no PAS-positive granules were present.

(8) *Kitten.*—In a 5-day-old normal control kitten (K4/2), PAS-positive granules distributed in a diffuse fashion were found in relation to the developing vessels. This observation was repeated in several other normally developing kittens.

In the kittens in which new vessel formation in the retina and vitreous had been induced by treatment with oxygen (as described by Ashton and others, 1953, 1954), PAS-positive granules were found in the retina in relation to proliferating cell masses but not in the vitreous. In addition, where they occurred intra-retinally, the density of the granules was greater in relation to proliferating cell masses than to newly formed canalized capillaries. These findings are similar to those described in early retrolental fibroplasia in man.

Of interest is the fact that, in kittens which died of infections and were found dead in the cage, the retinae showed evidence of *post-mortem* degeneration and no PAS-positive granules were present.

Nature of Polysaccharide Granules

A summary of the histochemical observations made is given in Table IV (opposite) and Table V (overleaf). The significant findings are these:

(1) *Granules staining with PAS.*—These were found in the retina in early retrolental fibroplasia, in premature human eyes, in human foetal eyes (where vascularization had commenced), and in the eyes of new-born rabbits and kittens.

(2) *Granules staining with Iodine.*—These were found in the retina in early retrolental fibroplasia and in the 14-day-old rabbit.

(3) *Diastase Lability of these Granules.*—This was demonstrated in retrolental fibroplasia (Figs 6 and 7, opposite), in premature human eyes, and in the eyes of new-born rabbits and kittens.

TABLE IV
DIFFERENTIAL STAINING OF POLYSACCHARIDE GRANULES (R.L.F. 30 L)

Stain	Observation	Conclusion
PAS	Large numbers of PAS-positive granules in inner retinal layers related to proliferating mesenchymal cells	Granules are polysaccharide and may be of the following nature: glycogen, muco-polysaccharide, glycoprotein, mucoprotein, glycolipoid
Iodine	Most granules stained positively, but fewer than with PAS	Those which stain with iodine are glycogen in nature
Toluidine blue	No granules demonstrated	Not mucopolysaccharide in nature, *e.g.* hyaluronic acid, ester-sulphuric acid
Best's carmine	No granules demonstrated	Not glycogen in nature
Sudan black	No granules demonstrated	Not glycolipoid in nature
Southgate muci-carmine	No granules demonstrated	Not mucin in nature
Schiff's reagent without previous treatment with periodic acid	No granules demonstrated	No free aldehyde groups present

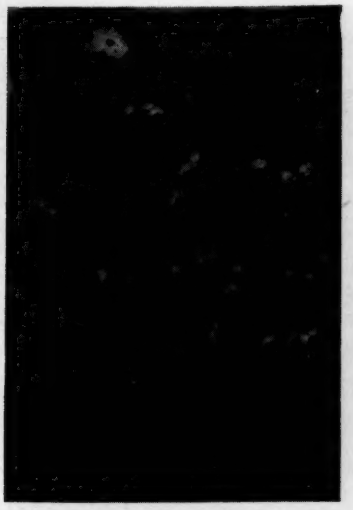

FIG. 6.—Enzyme study on RLF 30 L. Boiled diastase at 37° C. for 24 hrs had no effect on the PAS-positive granules. PAS and haematoxylin. ×520.

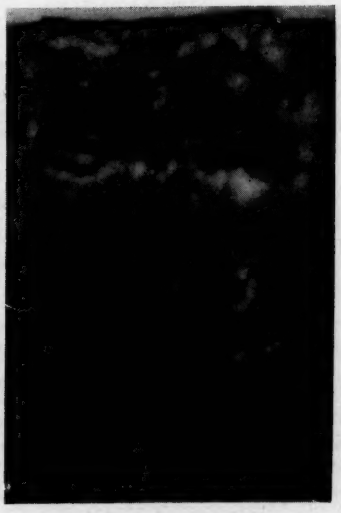

FIG. 7.—Enzyme study on RLF 30 L. Active diastase at 37° C. for 24 hrs has removed PAS-positive granules from specimen (Cf. Fig. 6). PAS and haematoxylin. ×520.

TABLE V
ENZYME STUDIES

Tissue	Enzyme	Buffer	Temperature (°C.)	Time (hrs.)	PAS-positive Granules	Conclusions
RLF 30L	HA	Acetate *p*H 4.4	37	24	+	Granules hyaluronidase stable, hence not mucopolysaccharide in nature, *i.e.* hyaluronic acid, or ester-sulphuric acid
	HB				+	
	HA			48	+	
	HB				+	
	HA		56	24	+	
	HB				+	
	DA	Saline	37	24	−	Granules diastase labile, hence glycogen in nature
	DB				+	
	DA			48	−	
	DB				+	
	DA		56	24	−	
	DB				+	
PM 126 R	DA	Saline	37	15	−	Granules diastase labile, hence glycogen in nature
	DB				+	
PM 126 L	DA	Saline			−	
	DB				+	
Rabbit (L)	HA	Acetate *p*H 4.4	37	5	+	Granules hyaluronidase stable, hence not mucopolysaccharide in nature
	HB				+	
	DA	Saline			−	Granules diastase labile, hence glycogen in nature
	DB				+	
Kitten (4/2)	HA	Acetate *p*H 4.4	37	24	+	Granules hyaluronidase stable
	HB				+	
	DA	Saline			−	Granules diastase labile, hence glycogen in nature
	DB				+	

HA—Active hyaluronidase HB—Boiled inactive hyaluronidase DA—Active diastase DB—Boiled inactive diataste
PAS-positive granules { + present, − absent
RLF 30 L—Specimen of early retrolental fibroplasia PM 126 L and R—Premature human eyes

Discussion

Occurrence of Polysaccharide Granules.—An intimate association of polysaccharide granules with the developing retinal vasculature, particularly with the proliferating mesenchymal cells of this system, is the main point which emerged from this investigation. The evidence for this association of granules with the developing vessels is as follows:

(1) No granules are found in human foetal retinae before the retinal vessels begin to develop.

(2) No granules are found in the retina in front of the most anterior limit of the developing retinal capillary bed, *i.e.* between this limit and the ora serrata. This is most apparent in man, but is true also of the rabbit and kitten.

(3) The granules are found in the inner retinal layers which are nourished by the retinal vessels. This is most obvious in the rabbit where the capillaries are found in the optic nerve fibre layer only.

(4) Granules are not found in the retinae of live-born full-term infants or in adults.

(5) At birth, neither the rabbit nor the kitten eye is mature, and PAS-positive granules occur in association with the developing retinal vessels at this stage of development in these animals.

(6) Because of the anatomy of the rabbit retina, no granules are found unless the section includes retinal vessels. The vessels are confined to the horizontal bands of medullated nerve fibres radiating into the retina from the optic disc.

In the development of the retinal vessels mesenchymal cell proliferation occurs with later differentiation to form the lining cells of capillaries. The particular association of the polysaccharide granules is with these proliferating mesenchymal cells; this is supported by the following evidence which emerges from the observations reported:

(1) Mesenchymal cells at the anterior limit of the retinal capillary bed are associated with the maximal density of polysaccharide granules. This is supported by two sets of results:

(*a*) In live-born premature infants (Table I), 23 retinae showed mesenchymal cells occurring as well-defined masses, *i.e.* of Group C, D, or E (Fig. 1). PAS-positive granules were present in nineteen of these retinae, showing Group B or C distribution (Fig. 1).

(*b*) Of 25 stillborn premature infants, a co-existence of mesenchymal cell increase and PAS-positive granules at the anterior limit of the retinal capillary bed was found in 23 (Table III). Similar observations were made on the 16, 18, and 19 weeks' foetuses.

(2) Mesenchymal cells other than those at the anterior limit of the vascular bed are associated with polysaccharide granules.

In the group of live-born premature infants (Table I), if the thirteen retinae showing Group D and E distribution of mesenchymal cells are considered together, PAS-positive granules of Group C and D (Fig. 1) were found in nine cases.

(3) Mesenchymal cell proliferations in early retrolental fibroplasia are associated with large numbers of polysaccharide granules in the retina.

Of interest in relation to early retrolental fibroplasia are the three retinae (Table I) showing Group E distribution of mesenchymal cells (Fig. 1). This histological picture merges imperceptibly into that of early retrolental fibroplasia. These three retinae showed Group C distribution of PAS-positive granules, *i.e.* a diffuse distribution with maximal density anteriorly (Table I). It is important that in retrolental fibroplasia the polysaccharide granules are related to intra-retinal mesenchymal cell proliferations: where invasion of the vitreous occurs, granules are found in the adjacent retina only.

It is thus clear that the essential relationship is between mesenchymal cells and polysaccharide granules, and that this secondarily determines the relationship of the latter to the developing retinal vessels.

Nature of Polysaccharide Granules.—Polysaccharides of varying types, which have been extensively studied histochemically, are widely distributed throughout plants and animals in association with many cellular activities (Pearse, 1949, 1953).

Histochemical observations recorded in this paper demonstrate that the granules associated with the developing retinal vessels consist of glycogen. Several points emerge from these observations for further discussion:

(1) All the granules were diastase labile and some stained positively with iodine.

Histochemically, diastase lability is regarded as a more specific test for glycogen than the old empiric staining methods with iodine and Best's carmine (Pearse, 1953, and others). No explanation can be offered why the granules did not stain with Best's carmine or why only some stained with iodine (Table IV). Bembridge and Pirie (1951) found that polysaccharide granules in the rabbit stained with Best's carmine but not with iodine, and concluded that the retinal polysaccharide was glycogen. We agree with this, but the demonstration of diastase lability is more conclusive.

(2) All tests for other polysaccharides were negative.

(3) The appearance of the polysaccharide as granules in a tissue fixed in formol saline suggests its glycogenic nature.

Since Bernard (see Creighton, 1896) observed glycogen microscopically by using iodine, opinions have varied as to its water solubility in tissues and hence as to the best method of fixation for its histochemical demonstration.

Bernard used a non-aqueous fixative and stained with an alcoholic iodine solution; on the other hand, Creighton (1896) used an aqueous iodine solution for staining material fixed in potassium bichromate and alcohol. Both authors described the glycogen as occurring in granular form.

Using the freeze-drying technique for fixation, Bensley and Gersh (1933) demonstrated a homogenous staining of cytoplasm, whereas with other methods, (e.g. chemical fixation with alcohol or formalin), various degrees of granularity were demonstrated with glycogen stains. This granular appearance is in fact an artefact (Gersh, 1932; Lazarow, 1942), and as our material was fixed in 10 per cent. formol saline, an explanation for the occurrence of the glycogen in granular form is apparent. With regard to the fixation of retinal glycogen in sections, Bembridge and Pirie (1951) state that formalin fixation did not preserve it as well as Bouin's fluid or 70 per cent. alcohol, and that this was probably due to the greater water content of the formalin (with loss of glycogen due to its water solubility). Kojima and Nagao (1953) also stated that alcohol fixation gave the best results. In our experience, however, fixation with 10 per cent. formol saline has been quite satisfactory. Recent work by Vallance-Owen (1948) and Carpenter and others (1951) has shown that formalin fixation followed by prolonged washing of the fixed block in water does not impair the staining of glycogen, and hence it appears that the water solubility of glycogen in the test tube is of no significance when glycogen in tissues is being considered. The water content of Bouin's fluid and of 10 per cent. formol saline is almost the same.

Significance of Glycogen Granules.—In any scheme of morphogenesis there are three components: cell proliferation and growth, differentiation of cells, and associated metabolic activity (Needham, 1942). Whilst the exact significance of glycogen granules is as yet unknown, it would appear that, in the development of the retinal vessels, their occurrence is an index of some transient metabolic activity. This is supported by observations of waves of mesodermal cell activity associated with glycogen granules inthe developing cornea, choroid, mesodermal iris, and pupillary membrane (Serpell, 1953).

A theory was put forward by Vastarini-Cresi (1921, 1924) that canalization of vessels and spaces is especially associated with glycogen metabolism. He stated that active cell proliferation to form buds was followed by glycogenic infiltration of the central cells of the bud. Later, cytolysis of these central cells occurred, resulting in the formation of a lumen. However, he did not indicate the exact metabolic significance of the glycogen.

Whilst these metabolic questions remain unanswered, the present study of glycogen in association with the developing retinal vessels provides an

explanation of the polysaccharide granules found in the retina in early retrolental fibroplasia, which is essentially a disease process of the immature retinal vasculature.

Summary

(1) Polysaccharide granules have been demonstrated in association with areas of increased mesenchymal cell content in the inner retinal layers in the early stage of retrolental fibroplasia.

(2) Similar granules occur in relation to the developing retinal vessels in man, the cat, and the rabbit.

(3) The relationship of polysaccharide granules to the developing retinal vasculature depends essentially on their association with proliferating mesenchymal cells in the developing vascular bed.

(4) The granules have been identified histochemically as glycogen.

(5) The metabolic significance of the glycogen granules associated with developing retinal vessels is not yet determined.

I wish to express my thanks to Dr. Norman Ashton for help and encouragement in this investigation and for advice in the preparation of this paper. I am also indebted to Mr. A. Johnston and Mr. G. Knight for technical assistance, and to the Department of Medical Illustration at the Institute of Ophthalmology, for the photographs.

REFERENCES

ASHTON, N. (1954). *British Joural of Ophthalmology*, **38**, 385.
——, WARD, B., and SERPELL, G. (1953). *Ibid.*, **37**, 513.
—— —— —— (1954). *Ibid.*, **38**, 397.
BEMBRIDGE, B. A., and PIRIE, A. (1951). *Ibid.*, **35**, 784.
BENSLEY, R. R., and GERSH, I. (1933). *Anat. Rec.*, **57**, 205, 217, 369.
BERNARD, C. *Quoted by* Creighton (1896).
BEST, F. (1903). *Beitr. path. Anat.*, **33**, 585. (*Quoted by* Cameron, 1952).
—— (1906). *Z. wiss. Mikr.*, **23**, 319.
BRAMMERTZ, W. (1915). *Arch. mikr. Anat.*, **86**, 1. (*Quoted by* Bembridge and Pirie, 1951).
CAMERON, G. R. (1952). "Pathology of the Cell". Oliver and Boyd, Edinburgh.
CARPENTER, A., POLONSKY, B., and MENTEN, M. L. (1951). *Arch. Path.* (*Chicago*), **51**, 480.
CREIGHTON, C. (1896). "Microscopic Researches on the Formative Property of Glycogen". Black, London.
DAY, R. (1950). *Amer. J. Ophthal.*, **33**, 224.
EHRLICH, B. (1883). *Cited by* Frerichs (1883).
FRERICHS, F. T. (1883). *Z. klin. Med.*, **6**, 33.
FRIEDENWALD, J. S. (1949). *Amer. J. Ophthal.*, **32**, 487.
GERSH, I. (1932). *Anat. Rec.*, **53**, 309.
HOTCHKISS, R. D. (1948). *Arch. Biochem.*, **16**, 131.
KOJIMA, K., and NAGAO, Y. (1953). *Acta soc. ophthal. jap.*, **57**, 127. (*Ophthal. Lit.* (*Lond.*), **7**, 94).
LAZAROW, A. (1942). *Science*, **95**, 49.
MCMANUS, J. F. A. (1946). *Nature*, **158**, 202.
—— (1948). *Stain Technology*, **23**, 99.
NAKASHIMA, C. (1926). *v. Graefes Arch. Ophthal.*, **116**, 403. (*Quoted by* Bembridge and Pirie, 1951).
NEEDHAM, J. (1931). "Chemical Embryology". University Press, Cambridge.
—— (1942). "Biochemistry and Morphogenesis". University Press, Cambridge.
PEARSE, A. G. E. (1949). *J. clin. Path.*, **2**, 81.
—— (1953). "Histochemistry". Churchill, London.
SCHMITZ-MOORMAN, P. (1927). *v. Graefes Arch. Ophthal.*, **118**, 506.
SERPELL, G. (1953). Personal observations.
VALLANCE-OWEN, J. (1948). *J. Path. Bact.*, **60**, 325.
VASTARINI-CRESI, J. (1921). *Atti. Accad. Med.-Chir. Napoli.*, **75**, 1.
—— (1924). *Arch. ital. Biol.*, **73**, 97.
WARD, B. (1954). *British Journal of Ophthalmology*, **38**, 445.
WOLFF, E. (1948). "The Anatomy of the Eye and Orbit", 3rd ed. Lewis, London.

Brit. J. Ophthal. (1954), 38, 472.

VASCULARIZATION OF THE OPTIC PATHWAY*

I. LAMINA CRIBROSA AND OPTIC NERVE

BY

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THIS problem has been of only moderate interest to research workers, and publications and monographs on the subject have been sporadic, though a few well-known names must be cited here (Leber, 1865a, b, 1872, 1903; Magitot, 1908, 1947; Behr, 1935; Wolff, 1939, 1940, 1948). The findings being very diverse, it seemed appropriate to try to find experimentally the normal vascular anatomy of the optic pathway, especially because alterations in the vessels play a preponderant role in the visual system.

The finer details of the circulation are practically unknown, less and less data being available as we approach the capillary stage. The capillaries are definitely nutritive, being responsible for metabolism in normal physiological conditions, and even in certain pathological processes.

Embryology and comparative anatomy have been hitherto neglected in the explanation of the experimental results, but the chronology of the embryological processes is very important in providing objective proofs of anatomical conceptions.

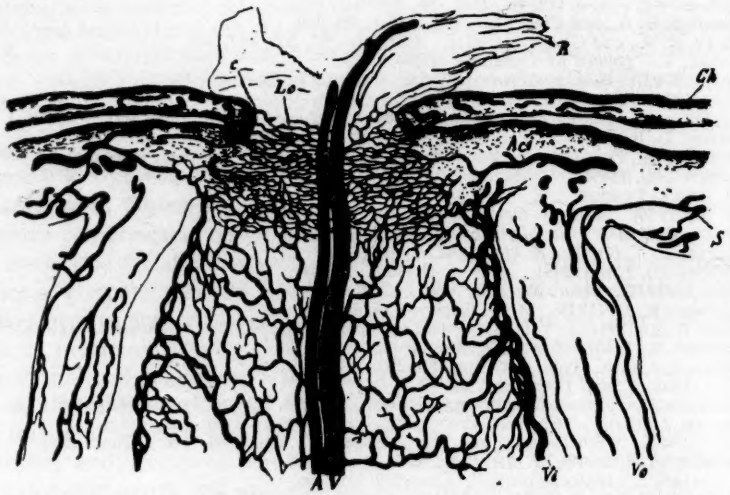

FIG. 1.—Vascularization of optic nerve and lamina cribrosa, according to Leber (1903).

S	sclera	A	central retinal artery
Ch	choroid	Lc	lamina cribrosa
R	retina	Aci	short posterior ciliary artery which sends a branch to the optic nerve
Ve	vessels of dura		
Vi	vessels of pia	C	anastomoses between choroid and optic nerve

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Historical Survey

The first investigation of the vascularization of the optic nerve and the eye was performed by Zinn (1755) and Haller (1754). To them is credited the description of numerous branches arising from the short posterior ciliary arteries to build up in the sclera, surrounding the optic nerve, an anastomotic network, resulting in a closed circle: the scleral capillaries which, according to Zinn and Haller, form anastomoses with the capillaries of the anterior part of the optic nerve and with branches from the central artery of the retina. Tiedemann (1824) and Huschke (1844) later described them again. Jaeger (1861) fixed attention on this specialized vascular system, which takes an important part in pathological changes at the level of the optic nerve and its surroundings, emphasizing that the system is not closed at all, branches being given off to the choroid, the retina, and the optic nerve.

The optic nerve was considered by Zinn and Haller to be supplied by the central retinal artery alone.

The ideas put forward by Leber (1865, 1872, 1903), concerning the vessels of the eye and the optic nerve are still of interest (Figs 1 and 2).

The short posterior ciliary arteries, dividing into fine branches, feed the choroid. At the surface of the sclera, they give off branches, resulting in a network with wide meshes, which form ramifications, nourish the posterior sclera, and, after reaching the pia mater, penetrate into the optic nerve. The smallest of these anastomose in the sclera and, at the level of the optic nerve, build up several concentric arcs connected by radial branches, which proceed in a network of irregular meshes

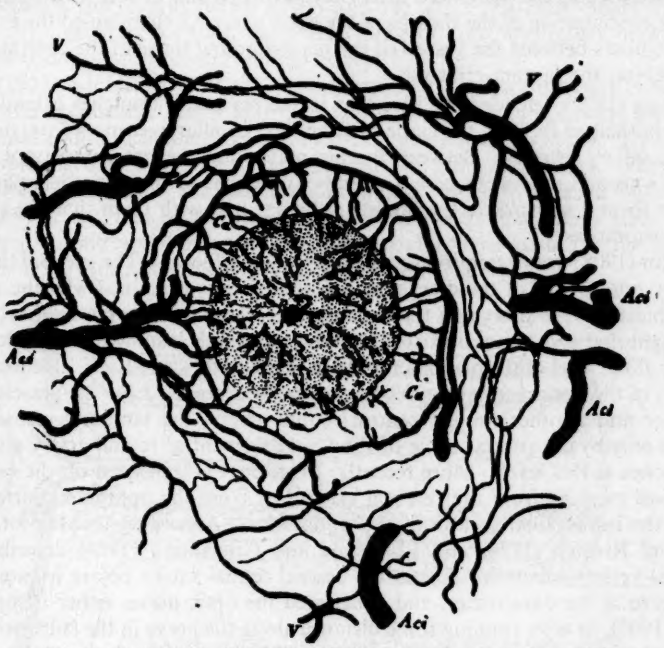

FIG. 2.—Arterial circle of Zinn-Haller, according to Leber (1903).

Aci	short posterior ciliary arteries	a	central retinal artery
V	short posterior ciliary veins	v	central retinal vein
Ca	Zinn-Haller's arterial circle		

placed perpendicularly to one another: the arterial circle of Zinn. As it extends forwards, this network diminishes. The arterial circle sends branches to the optic nerve, choroid, and retina, and the most developed of these becomes the ciliary retinal arteries. From the choroid, numerous branches arise to supply the optic nerve and the lamina cribrosa, *via* the pia mater. The lamina cribrosa is also supplied by ramifications of the central retinal artery, anastomosing with the circle of Zinn.

The optic nerve is nourished by the central retinal artery; the portion posterior to its point of entry being supplied by branches arising from the pia mater. It also gives rise to a branch, which divides in two: one running up anteriorly and the other in the direction of the optic foramen, both being situated in the axis of the optic nerve (Vossius, 1883).

The intracranial part of the nerve is supplied by the ophthalmic artery and the corpus callosum artery, and the orbital part by the ophthalmic artery alone.

Elschnig (1893) inclined to the view that there are no anastomoses between the central retinal artery and the circle of Zinn.

According to Leber (1865a, b), the optic nervehead is dependent upon the central retinal artery; this is contrary to the opinion of Galezowski (1865) who put forward the view that the vessels of the papilla of the optic nerve derived from the pia mater and were of cerebral origin. Again, according to Leber, the circle of Zinn plays the role of the dura and pia mater vessels of the optic nerve (cf. Hyrtl, 1839: the vaginal artery, and the interstitial artery respectively), and in this way the circle of Zinn is a continuation of the sheaths of the optic nerve. Leber denied the existence of anastomoses between the vessels of the pia mater and those of the central retinal artery behind the lamina cribrosa.

Wolfring (1872) defended the idea that in the pia mater branches extended forwards, and helped to form the circle of Zinn with capillaries coming from the short posterior ciliary arteries. Between the pia mater and the optic nerve are vessels forming a second meshwork which extends to the axis of the optic nerve and forwards to form a network in the lamina cribrosa: it is with them that the circle of Zinn anastomoses.

Magitot (1908) drew attention to an embryological factor. The eye and the optic nerve are outgrowths of the diencephalon, and he thus explained why the arteries to the chiasma were short and those to the globe and optic nerve relatively long. He thought that anastomoses existed between the vessels leading to the optic nerve, but only at the level of the pia mater and not in the optic nerve itself. He wondered whether, in the optic nerve, one definite region was reserved for the vessels of the pia mater and another for the central retinal artery, the lamina cribrosa being supplied only by the arterial circle of Zinn, and the central retinal artery giving off no branches at this level. More recently, Magitot (1947) insisted on the existence of a vessel running from the cerebral arteries or from the ophthalmic artery and feeding the intracranial section of the optic nerve. According to Magitot, Beauvieux and Ristitch (1924) and Beauvieux and Gouelmino (1924) described five collateral vessels which arose from the central retinal ratery before it entered the optic nerve in the dura mater, and penetrated the optic nerve, either immediately (Leber, 1903), or after running some distance along the nerve in the subarachnoidal space. Having once entered the optic nerve, the central retinal artery gives off no more branches; anastomoses with the other networks do not exist. Proximal to

the point of entry of the central retinal artery, the ciliary arteries help to build up the network of the pia mater, and distal to it branches supply the optic nerve *via* the pia mater. According to Magitot these branches play a part in feeding the lamina cribrosa (Fig. 3).

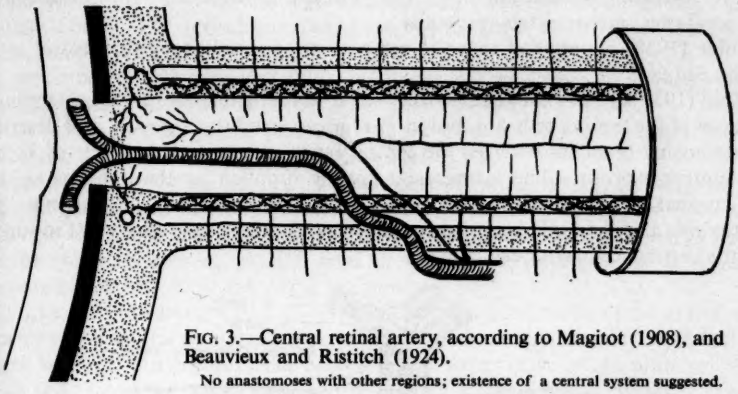

FIG. 3.—Central retinal artery, according to Magitot (1908), and Beauvieux and Ristitch (1924).
No anastomoses with other regions; existence of a central system suggested.

Rochon-Duvigneaud (1933) postulated the existence of two kinds of anastomoses between the central retinal artery and the short posterior ciliary arteries: indirect, through branches from the arterial circle of Zinn, and direct, between the choroidal vessels and the central retinal artery.

Behr (1935) established that the optic nerve in its intracranial part is supplied by both the ophthalmic artery and the anterior communicating artery, and in its intra-orbital and intracanalicular parts by the ophthalmic artery alone. He thought that the lacrimal artery might also assist in nourishing the optic nerve. The central retinal artery gives off in the anterior and middle thirds an anterior optic nerve (para-axial) artery, and in the posterior third the ophthalmic artery gives rise to the posterior optic nerve arteries. Thus an axial system exists for the anterior section, and may also exist for the posterior section (Fig. 4).

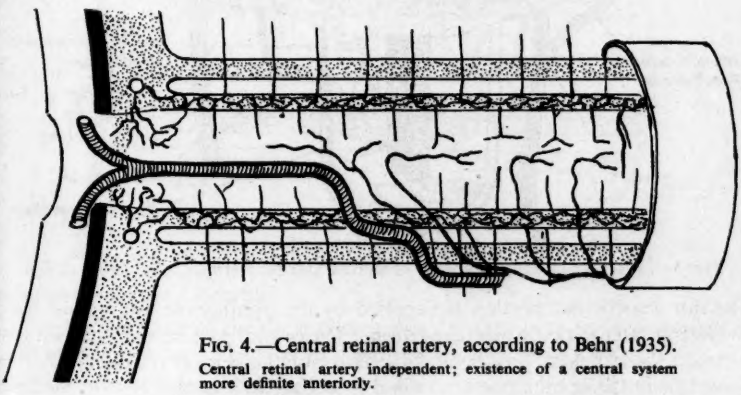

FIG. 4.—Central retinal artery, according to Behr (1935).
Central retinal artery independent; existence of a central system more definite anteriorly.

The lamina cribrosa is supplied by branches of the arterial circle of Zinn and by the central anterior optic nerve artery, which may give off capillaries serving as vasa vasorum for the central retinal artery, which gives off no branch in the optic nerve itself. The anterior part is also nourished by branches of the central retinal artery penetrating into the pia mater, that is to say, centripetally. In the optic nerve all capillaries anastomoses mutually.

Abbie (1938) maintained that the optic nerve is supplied by the central artery of the retina.

Wolff (1939) did not attribute to the central retinal artery a capillary extending at the level of the lamina cribrosa, but in 1940 he reversed this opinion, and described anastomosing branches between the central retinal artery and the circle of Zinn in the lamina cribrosa. The intracranial part is supplied by the anterior cerebral and internal carotid arteries in the superior and inferior aspects respectively. The ophthalmic artery and the anterior communicating artery may also assist in supplying the central retinal artery (Fig. 5).

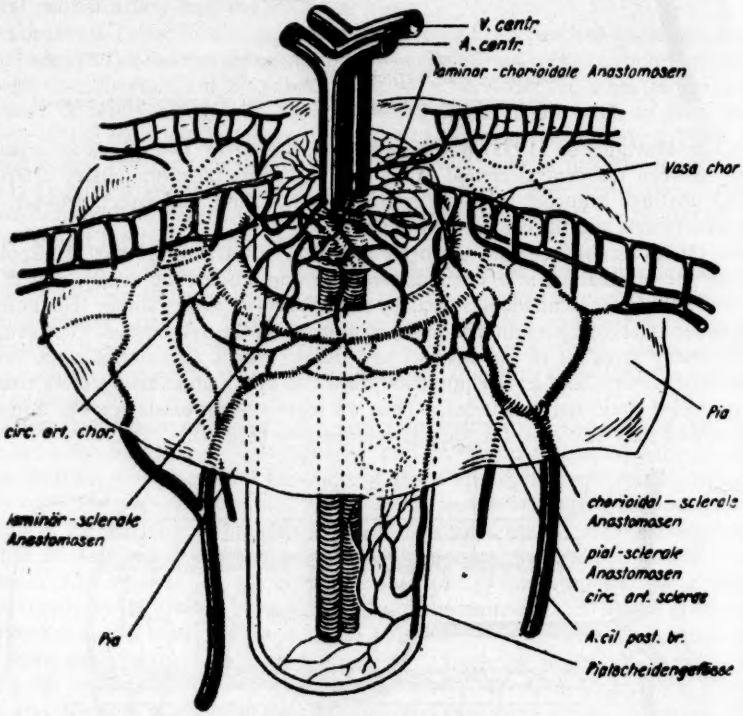

FIG. 5.—Vascular system, according to Sautter and Seitz (1952), after Leber (1903).

The intracanalicular portion is supplied by the ophthalmic artery, but the pial network is poorer than the orbital portion. The orbital part depends on two groups of vessels: those penetrating behind the entry of the central retinal artery, which originate from the ophthalmic artery and its collateral branches (less in the inferior

aspects), and those which are themselves branches of the central retinal artery (Kuhnt, 1879). These were described again by Beauvieux and Ristitch (1924) as collateral central retinal arteries penetrating into the optic nerve *via* the pia mater network. One of these arteries accompanies the central retinal artery to the centre of the optic nerve and divides into anterior and posterior branches feeding the macular fibres. The nerve head is supplied by the central retinal artery and by the arterial circle of Zinn.

Igersheimer (1942) supposed that the central retinal artery might occasionally give off branches with a nutritive purpose behind the lamina cribrosa.

Kiss (1943) mentioned the possibility of the existence of anastomoses between the central retinal artery and the arterial circle of Zinn.

Duke-Elder (1938) maintained that the central retinal artery, running forward and axially, gives off numerous small recurrent branches to supply the optic nerve up to the optic foramen, and then branches to the other parts of the nerve and its sheaths along its course. At the level of the lamina cribrosa several capillary anastomoses exist within the circle of Zinn.

Traquair (1946) thought that no vessel pierced the dura mater distal to the point of entry of the central retinal artery, and that all pial vessels derived from the central retinal artery in this region. The central retinal artery gave off no branches, once it had penetrated the optic nerve, except for the lamina cribrosa, where it anastomosed with the circle of Zinn.

Sautter and Seitz (1952) concluded experimentally that Leber's idea of the lamina cribrosa was correct: it was supplied by the central retinal artery, and there existed, moreover, functional anastomoses between the circle of Zinn and the central retinal artery; this was achieved by glomic cells situated close to the capillaries, which might also have a biochemically antitoxic function. Their experiments were performed on living rabbits. We did not find these glomic cells on the numerous slides we examined, and we are not at all certain of the existence of any central retinal artery in the rabbit (Fig. 6).

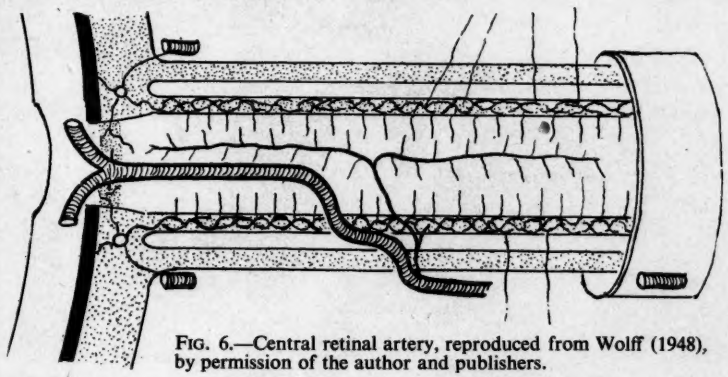

FIG. 6.—Central retinal artery, reproduced from Wolff (1948), by permission of the author and publishers.

Conclusions from the Literature

(1) For the nutrition of the optic nerve from the outside to the axis (centripetal) the pial vessels give rise to capillaries penetrating to the nerve itself.

(2) The short posterior ciliary arteries, before running into the choroid, give off

branches building up the arterial circle of Zinn-Haller, from which capillaries ramify into the lamina cribrosa.

(3) Capillary anastomoses may exist between the central retinal artery and the arterial circle of Zinn, which carry out both nutritional and biochemically antitoxic functions.

(4) The central retinal artery supplies the optic nerve through collateral branches *via* the pia mater, through branches immediately directed to the optic nerve, and through branches given off immediately before, or after penetrating into the optic nerve and lying in the axis; these form a source of nourishment from within to the periphery (centrifugal).

(5) The central retinal artery may give off branches only outside the optic nerve.

(6) Anastomoses may exist, not only between the pial vessels but also at the capillary stage, between the capillaries of one system, and between those of the axial and peripheral systems.

(7) The intra-orbital supply of the optic nerve is probably independent of the intracranial supply.

(8) The lamina cribrosa may be supplied by the circle of Zinn alone, or by the central retinal artery alone, or by both.

(9) The vessels of the optic nerve derive from the central retinal artery and the ophthalmic artery, and the only source of the blood is the internal carotid artery.

(10) The region of supply of the central retinal artery is not yet established.

(11) Galezowski (1865) was the first and only writer to draw attention to the existence of capillaries of directly cerebral origin at the level of the optic nerve, lamina cribrosa, and papilla.

(12) Wolfring (1872) noticed anastomoses in the lamina cribrosa between the circle of Zinn and capillaries coming from behind the lamina cribrosa; he supposed that the latter derived from a secondary network between the pia mater and the optic nerve.

(13) Magitot (1947) was more definite than Leber (1903) and Behr (1935) in excluding the existence of anastomoses; Leber agreed with the concept of anastomoses at the level of the lamina cribrosa only, and Behr with the concept of anastomoses in the optic nerve only.

Discussion of the Literature

(1) Three points, taken from the literature, require elucidation:

(*a*) The significance of the capillaries of cerebral origin observed by Galezowski (1865).

(*b*) The possibility of anastomoses in the lamina cribrosa with capillaries from behind this structure (Wolfring, 1872).

(*c*) The disagreement on the subject of anastomoses; no two authors agree on the existence of an axial system, or about the behaviour, relations, and distribution of the central retinal artery, the circle of Zinn, and the pial vessels.

(2) The central retinal artery penetrates into the optic nerve between 10 and 20 mm. behind the globe, but to supply the optic nerve over a length of 45 mm., having once penetrated into and run inside the nerve over a distance of only 15 mm., is a different matter. The central retinal artery can give off branches in its extra-neural

course, but this is at the inferior aspect of the nerve, and it is this part which is relatively poorly supplied; some fortuitous branches may always exist, but they cannot be considered as taking the leading role in nutrition. This is indicated by comparative anatomy and by our own experiments (See below).

(3) The central retinal artery divides on the papilla, in its end-branches, just behind, or in the lamina cribrosa, or even in the optic nerve itself. It has been recorded experimentally and also by us that these branches never form anastomoses. They always supply strictly retinal tissues. The existence of capillary anastomoses between the circle of Zinn and the central retinal artery in cases where the central retinal artery divides just behind the lamina cribrosa, is difficult to credit; the cases recorded by Igersheimer (1942) may have been of this kind.

(4) The fact that the central retinal artery always retains the same calibre from its origin to its division, argues against the existence of collateral vessels of any importance.

(5) The capillaries of the circle of Zinn anastomose with other capillaries. Normally there is a transition to the venous side of the capillary system, but the venous circulation in the circle of Zinn seems to be absent, and thus it is necessary for these capillaries to join other vessels possessing the normal relations. The anastomoses offer an easier outlet for the blood.

Before coming to the last three sections of this study (embryology, comparative anatomy, and our own experiments) a few facts should be re-called:

(1) Ordinarily there exists a large margin of variation, based on embryology, for the course of the arteries before they reach the region they must supply: but there can be little variation in the region supplied.

(2) For the neural tissues of the brain and those depending on it, it is not true that arterial anastomoses are impossible and that they are all end-arteries.

(3) Whether this is true for the capillaries, and whether a rete mirabile may be admitted for the optic nerve remains an open question.

Embryology

Up to the third week after the fertilization of the ovum (the 3 to 4 mm. stage) a vascular mesoderm of one layer of cells begins to appear with nucleated erythrocytes.

In the fourth week the optic cup is formed and the foetal fissure can be seen, on the temporal and inferior side of the distal part of the optic stalk, extending up to the brain. The fissure is filled up by mesoderm which penetrates immediately into the cup. From the beginning of their development the retina and the optic stalk are completely avascular.

At the 7-mm. stage (4th-5th week) the internal carotid artery curves over the optic stalk and gives off the ophthalmic artery from which arises the hyaloid artery; it penetrates the foetal fissure and the cup, and in the 5th week is buried by the closure of the lips of the fissure. This hyaloid artery gives off no branches, either the optic stalk, or to the retina.

Not the smallest trace of a choroidal network of vessels is to be found, except the condensation of some small capillaries here and there just in front of those parts of the external wall of the optic cup which take up pigment.

Later still the vessels of the anterior cerebral plexus are built up on the

temporal side of the prosencephalon almost as far as the diencephalon.

Small vessels begin to be distinguished in the orbital mesoderm and help to form the vascular system which will later evolve in the pia mater and near the posterior pole of the globe, and those parts which will later become the orbit. They communicate with the anterior cerebral plexus.

The foetal fissure is closed by the 6th week (12-mm. stage) except where the hyaloid artery penetrates; but at 17-mm. stage the marginal border is obliterated.

But even before this, the ophthalmic artery gives off the ciliary artery on the temporal side of the optic stalk, wherein the axons of the retina start to penetrate.

The mesodermal sheaths of the optic nerve are now formed (17-mm. stage), and immediately afterwards (19-mm. stage) the glial septa are formed by the inner layer of the optic stalk. The hyaloid vessel proper is now in existence.

During the 7th week the anterior ciliary arteries arise from the ophthalmic artery, and the common ciliary artery divides into the long temporal posterior ciliary artery and the long nasal posterior ciliary artery. The latter divides into twenty short posterior ciliary arteries during the 8th week (20-mm stage).

The first condensation of the sclera from the surrounding mesoderm occurs in the 9th week and appears at the posterior pole of the globe.

Between the 2nd and 3rd month (30-mm. stage), the short posterior ciliary arteries reach the vascular choroid; the septa of the optic nerve do not yet exist.

At the level of the lamina cribrosa, glial tissues always predominate (continuations of Bergmeister's papilla).

In the third month, at 12 to 13 weeks (65 to 73-mm. stage), vessels may be seen arising from the ophthalmic artery, accompanying the hyaloid artery for some distance, and then extending forwards, penetrating into the optic nerve by way of the sheaths and starting to supply it.

The hyaloid artery now begins to atrophy through the multiplication of glial cells of the papilla of Bergmeister around it, thus leaving a space between artery and glial sheath. This process proceeds from the proximal to the distal direction.

In the fourth month the bud of the retinal branches of the hyaloid artery becomes evident; this indicates the origin of the retinal branches of the intraneural, proximal segment of the hyaloid artery; it later becomes the central retinal artery. In this way a rupture, not anatomical but physiological, occurs between the intra-ocular and intraneural parts of the hyaloid artery. The retina now begins to be supplied by the proximal parts of the hyaloid artery, and simultaneously the optic nerve begins to be supplied by a definite tubular system. The central artery becomes functionally active in nourishing the retina, and the short posterior ciliary arteries build up the circle of Zinn (eventually developing into the cilio-retinal arteries), and feed the distal part of the optic nerve.

Meanwhile the vessels supplying the optic nerves have reached the region behind the lamina cribrosa, and ramifications probably penetrate and form connections with the circle of Zinn.

By the 7th month the hyaloid artery is not completely obliterated (but so is physiologically), and the medullation of the optic nerve has begun, though this has rarely reached the lamina cribrosa by birth. Elements of the choroid and fibres of the sclera penetrate into the choroidal lamina cribrosa, carrying with them blood vessels to give the scleral lamina cribrosa its final composition.

The hyaloid artery, which first feeds the anterior parts of the optic cup and the lens, loses this function in order to take up its definite work: the supply of the retina by its proximal part, a quality seen only in the primates and in man. Anastomoses with the anterior cerebral vessel may persist.

This process of embryological evolution confirms our experimental ideas. Indeed, during the development of the foetus, the central retinal artery supplies the retina only; the circle of Zinn and the other arterial branches of the ophthalmic artery supply only the optic nerve, and the various periods at which the different systems develop and reach their particular regions indicate that some of them are entirely excluded from nourishing the optic nerve.

Comparative Anatomy

The hyaloid artery is always present in mammals, but tends to disappear later than in man.

It is present at birth in the cat, and disappears only 1 or 2 months after birth. In the mole, which has a very low degree of visual acuity, the hyaloid artery persists throughout life. The hyaloid artery always exists at the beginning; the longer it persists the more likely is the central retinal artery to be absent, and the sooner it goes (as in man) the greater is possibility that a central retinal artery will develop.

The vessels destined for the retina, if they exist, penetrate more nearly into the globe in the other mammals than in man, because in man the central retinal artery must be better attached and protected than in animals, for the higher visual acuity of the human retina to be achieved. The ciliary vessels are more important in animals, because they play the largest part in nourishing the retina. It is not certain that the dog, cat, and fox have any central retinal artery at all. In the dog the retina is supplied by cilio-retinal arteries, deriving from the circle of Zinn. When the central retinal artery is absent, and when the hyaloid artery has disappeared completely, the ciliary system is predominant. It has been established that in these animals central arteries exist in the optic nerve extending forwards by ramifications (Wolff, 1948; Davies, 1928). Bernoulli (1949) reported a rare case of the persistence of a hyaloid artery in a young boy with an anangiotic retina, and Holm (1923) another case with very small vessels in the retina.

We can conclude that the vessels of the optic nerve and the vessels of the circle of Zinn are the oldest, and that the central retinal artery has the special function in the higher mammals (the primates and man) of supplying the retina according to their more highly developed visual acuity. The human retina belongs both to the central artery type, and to the holangiotic type.

In man the nutrition of the retina and optic nerve is evenly shared, and the ciliary system is reserved for the choroid, lamina cribrosa, and partly for the optic nerve; whereas in animals the ciliary system has to supply the retina also, at least in paurangiotic, merangiotic, and holangiotic types of fundus. The central optic nerve artery seems to exist in animals as in man.

Experimental Work

Method.—The technique of studying the vascularization of the optic pathway had to be adapted to our special purpose, so that we might observe these vessels in the finest detail.

In the living man or animal we can proceed by arteriography, but by this technique only the greater vessels of the orbit and the brain are visible, and an *x*-ray film taken when the capillaries are filled with the contrast medium gives only a hazy picture.

We used *post-mortem* material by two different methods:

(1) Dissection of arteries and arterioles before and after fixation in formol 10 per cent.

(2) Injection of a plastic material, Neoprene latex 572, with additional colour. We used the apparatus and technique described by Ashton (1951) in his studies of Schlemm's canal. This method was used by Lieb (1940) and by Duff and More (1944), and later by Trueta and others (1947) in studies of the renal circulation.

The injected specimens were immersed in formol 10 per cent. and dissected, or soaked in pepsin and then in trypsin to digest the surrounding non-injected tissues and obtain a vascular tree. This is more difficult with the optic nerve and lamina cribrosa than with the retina and choroid, because the former tissues are more fibrous.

Material.—We injected a total of 34 specimens.

(1) *Ophthalmic Artery* (four times).—The whole tree was filled up, but the capillary regions were not all well filled.

(2) *Central Retinal Artery* (eight times).—The retina was very well injected, and also at times a few pial vessels.

(3) *Central Optic Nerve Artery* (six times).—The optic nerve was filled to the centre, and the lamina cribrosa completely.

(4) *Short Posterior Ciliary Artery* (once).—A small region of the choroid and some capillaries of the lamina cribrosa were filled. Although difficult to obtain, this injection did not teach us much.

We dissected with the binocular microscope after fixation in formol 10 per cent. and partial digestion. For the arteries and arterioles we dissected immediately after fixation or digestion, but the other specimens were either not well injected or were directly dissected without injection or fixation.

Neither after injection of the central retinal artery alone, nor after injection of the ophthalmic artery at a lower point in its course, nor after injection of one of the short posterior ciliary arteries, was it possible to show a clear communication between the central retinal artery and the arterial circle of Zinn. At first we thought that no anastomoses existed, or that the injection-mass did not penetrate into the finest capillaries.

The most interesting point was an obvious axial filling-up in the optic nerve, the origin of which remained obscure.

At one stage, while doing the preliminary dissection of the first collateral arteries of the ophthalmic artery, we detected a small artery, which we supposed to be the central retinal artery; we injected this vessel and filled all the axial system and the lamina cribrosa. After dissection we found the central retinal artery quite empty and the retina not injected. This vessel was injected five times, and gave the following anatomical picture:

A small collateral of the ophthalmic artery arising before the origin of the central retinal artery, extending forwards at the inferior temporal or inferior nasal aspect of the optic nerve (and crossing, in two cases out of six, the optic nerve below to the nasal side) entered into the optic nerve without having divided at all (or having divided into two branches only). Sometimes it entered near the point of entry of the central retinal artery, sometimes 5 to 10 mm. behind it, and it was guided to the axis of the nerve, where it divided (if this division did not already exist) into anterior and posterior branches. At the level of the lamina cribrosa it anastomosed with the capillaries of the circle of Zinn, but at the level of the optic foramen the anastomoses were doubtful. We were then sure that we were dealing with a central optic nerve artery, a collateral of the ophthalmic artery with anterior and posterior branches, the former anastomosing forwards with the circle of Zinn. The anterior branch lay on the temporal side and the posterior branch towards or even below the centre. This vessel appeared to be designed to supply the macular fibres, and the capillaries of the anterior branch might also have acted as vasa vasorum for the central retinal artery.

It was now clear why the injection into the ophthalmic artery at a point in its course lower than the origin of the central optic nerve artery did not fill the lamina cribrosa or the axial system.

When the central retinal artery is injected the retina is very well filled, but no other region becomes clearly evident. Sometimes a few injected small branches are found to derive from the central retinal artery and join the pial network outside the optic nerve. The vessels which run to the pia mater in the neighbourhood of the point of entry of the central retinal artery are not collateral arteries of the latter, but arise further back, coming from the ophthalmic artery, which can give off a recurrent branch to the chiasma, or from the central retinal artery just after its origin. The ophthalmic artery has recurrent branches anastomosing with branches from the internal carotid artery and branches of the anterior cerebral artery; from these, small anastomotic branch arteries also run to the pial sheath.

An axial system and a peripheral system exist in the optic nerve. In the pial network we could not find frequent anastomoses, and we could not establish whether anastomoses existed in the optic nerve itself.

An interesting experiment (although not very helpful in confirming the existence of anastomoses between the central optic nerve artery and the circle of Zinn) is to fill by injection a short posterior ciliary artery; this filling extends into the lamina cribrosa posteriorly and centripetally. After injection the branches issuing from the short posterior ciliary arteries run into the lamina cribrosa and extend forward to the choroid.

Fig. 7 (opposite) shows the arteries which help to supply the optic nerve and lamina cribrosa and also their anastomoses. Moreover, branches of the cerebral anterior artery can, after anastomosing with branches of the internal carotid artery, help to supply the optic nerve. The table also shows that, in conditions of changed position or in pathological processes (not only vascular), a retrograde nutrition *via* the anastomoses with the external carotid artery is certainly possible, besides that from the anterior communicating artery, the anterior cerebral artery, and the arterial circle of Willis.

Conclusions

(I) Lamina Cribrosa and Papilla (Fig. 8, overleaf).

(*a*) *Sclera*

(*i*) Capillary ramifications issue from the arterial circle of Zinn-Haller, built up by branches of the short posterior ciliary arteries. These ramifications anastomose with the terminal capillaries of the anterior branch of the central artery of the optic nerve, and not with the central retinal artery, which is strictly terminal and gives off no branches throughout its course, apart from some small ones to the pial network.

(*ii*) Ramifications of the short posterior ciliary arteries first supply the sclera, and then continue to the lamina cribrosa.

(*b*) *Nervous System*

(*i*) Branches issue from the vascular network of the pia mater.

(*ii*) Terminal ramifications of the anterior branch of the central optic nerve artery anastomose with those of the arterial circle of Zinn.

(*c*) *Choroid.*—Recurrent branches issue from the choroidal vessels.

(II) Optic Nerve (Fig. 9, overleaf).

(*a*) The central retinal artery passes *through* the optic nerve between 10 and 20 mm. behind the posterior pole of the globe.

(*b*) The optic nerve is supplied by capillaries issuing from the vascular network of the pia mater throughout its length. This network is composed of branches from the following arteries:

(*i*) from anastomoses between the recurrent collateral twigs of the ophthalmic artery and the collateral twigs of the cerebral part of the internal carotid artery.

(*ii*) from anastomoses between twigs of the anterior cerebral artery and twigs of the cerebral part of the internal carotid artery.

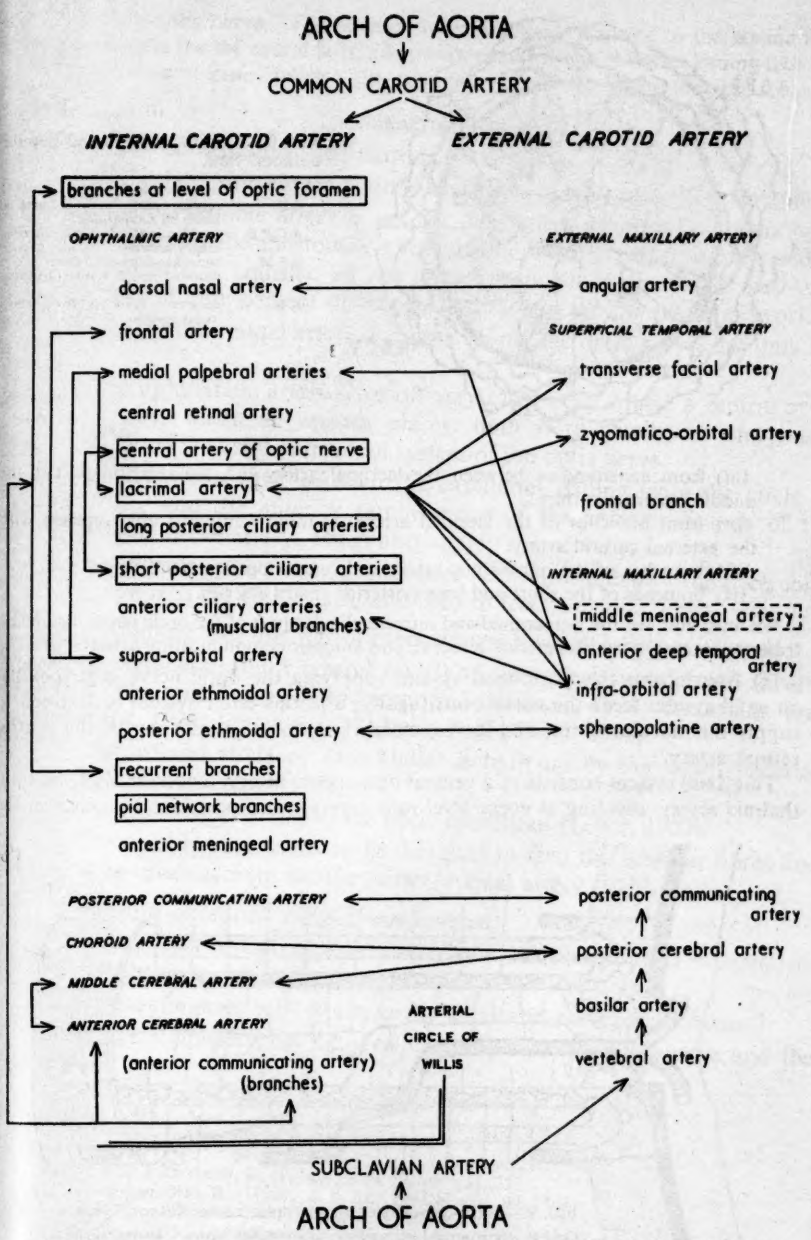

FIG. 7.—Diagram of optic nerve supply and anastomoses of arteries of visual pathway.

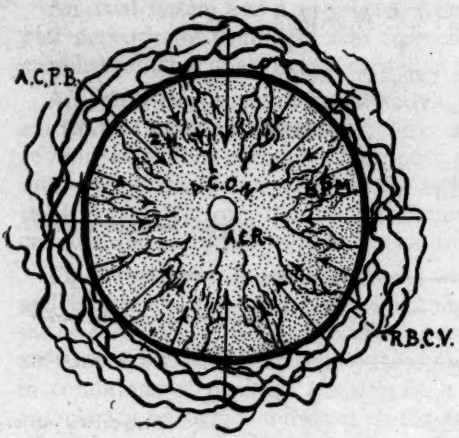

FIG. 8.—Lamina cribrosa and papilla, authors' view.

A.C.	central retinal artery
Z.H.	capillary ramifications of arterial circle of Zinn-Haller.
A.C.P.B.	ramifications of short posterior ciliary arteries
B.P.M.	branches from pia mater
A.C.O.N.	terminal ramifications of central optic nerve artery
R.B.C.V.	recurrent branches from choroidal vessels

(*iii*) from anastomoses between the lacrimal artery and the cerebral part of the middle meningeal artery.

(*iv*) from branches of the lacrimal artery, forming numerous anastomoses with the external carotid artery.

(*v*) branches of the ophthalmic artery and central optic nerve artery.

(*vi*) branches of the short and long posterior ciliary arteries.

We must add, for the intracranial and intracanalicular part of the optic nerve, branches, from the internal carotid, anterior cerebral, and anterior communicating arteries.

(*c*) Apart from this peripheral system supplying the optic nerve centripetally, an axial system feeds the nerve centrifugally; and this latter system is destined to supply the macular fibres, and serves, meanwhile, as vasa vasorum for the central retinal artery.

This axial system consists of a central optic nerve artery, a collateral of the ophthalmic artery, dividing at every level into anterior and posterior branches in the

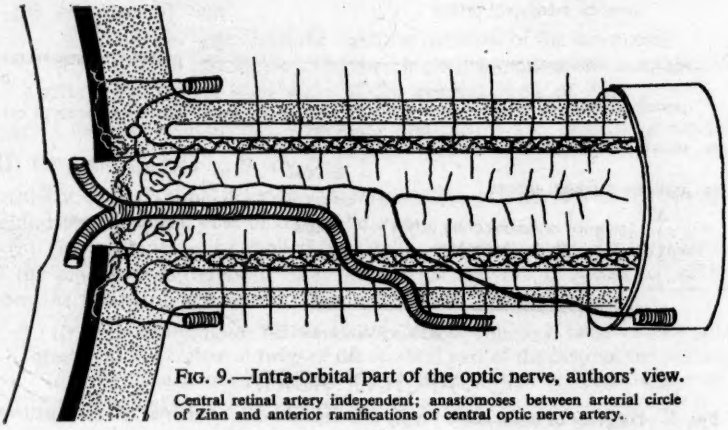

FIG. 9.—Intra-orbital part of the optic nerve, authors' view.
Central retinal artery independent; anastomoses between arterial circle of Zinn and anterior ramifications of central optic nerve artery.

axis of the optic nerve. The anterior branch extends forward to the lamina cribrosa, ramifies for the neural part, and anastomoses with capillaries issuing from the anterial circle of Zinn-Haller. The posterior branch extends to the optic foramen.

Summary of Findings

From these conclusions, the following facts emerge:

(1) The subdivision agreed upon is based upon embryological development.

(2) The ophthalmic artery is not the only vessel which supplies the optic nerve. Not only the anastomoses between the lacrimal artery and the middle meningeal artery, but also all the branches shown in the Table may contribute to the nourishment of the optic nerve *via* the pia mater network.

(3) The central retinal artery does not supply the optic nerve, but only the retina.

(4) The ophthalmic artery gives off very early in its course a central optic nerve artery, which at various places, both intra-canalicular and intra-orbital, forms the axial nutritional system of the optic nerve.

(5) Anastomoses exist between the capillaries issuing from the circle of Zinn-Haller and the anterior ramifications of the anterior branch of the central optic nerve artery, which also supply the nervehead.

(6) Capillaries issue from anastomoses between the extra-orbital cerebral vessels, run into the pial sheath, and help to feed the optic nerve.

Certain opinions and findings of other writers have also been confirmed:*

(1) A peripheral and a central (axial) vessel system (*via* the pial sheath) exist in the optic nerve (Beauvieux and Ristitch, 1924; Beauvieux and Gouelmino, 1924; Magitot, 1908; Behr, 1935; Wolff, 1948).

(2) The arterial circle of Zinn-Haller is built up by the short posterior ciliary arteries (Leber, 1903).

(3) Choroidal vessels run to the optic nervehead (Leber, 1903).

(4) The axial system seems to be designed to feed the macular fibres and to serve as vasa vasorum for the central retinal artery (Behr, 1935).

The following questions remain unanswered:

(1) Is the supply of the intracanalicular and intracranial parts independent of that of the intra-orbital part of the optic nerve?

(2) Do anastomoses exist between the axial and peripheral systems?

(3) Do anastomoses exist between the vessels of the optic nerve and the chiasma?

REFERENCES

ABBIE, A. A. (1938). *Med. J. Aust.*, **2**, 199.
ASHTON, N. (1951). *British Journal of Ophthalmology*, **35**, 291.
BARCLAY, A. E. (1951). "Micro-arteriography". Blackwell, Oxford.
BEAUVIEUX, J, and RISTITCH, K. (1924). *Arch. Ophtal.*, **41**, 352.
———, and GOUELMINO, R. (1924). *C.R. Soc. Biol. (Paris)*, **90**, 1241.
BEHR, C. (1935). *v. Graefes Arch. Ophthal.*, **134**, 227.
BERNOULLI, R. (1949). *Ophthalmologica (Basel)*, **117**, 169.

*We were not able to find the glomic cells described by Sautter and Seitz (1952).

CONE, W., and MACMILLAN, J. A. (1932). *In* " Cytology and Cellular Pathology of the Nervous System ", ed. W. Penfield, vol. 2, p. 837. Hoeber, New York.
DAMEL, C. S. (1936). *Arch. Oftal. (B. Aires)*, **11**, 57.
DUFF, G. L., and MORE, R. H. (1944). *J. techn. Meth.*, **24**, 1.
DUKE-ELDER, S. (1938). " Text-book of Ophthalmology ", vol. 1. Kimpton, London.
EISLER, P. (1930). " Kurzes Handbuch der Ophthalmologie ", ed. F. Schieck and A. Brückner, vol. 1, pp. 36, 135–158, 294–300, 492–493. Springer, Berlin.
ELSCHNIG, A. (1893). *v. Graefes Arch. Ophthal.*, **39**, pt. 4, 191.
FAWCETT, E. (1895). *J. Anat. (Lond.)*, **30**, 49.
FAZIO, C., and FARINA, P. (1940). *Riv. oto-neuro-oftal.*, **17**, 38.
GALEZOWSKI, X. (1865). *Gaz. hebdom. Méd. Chir.*, 2 sér., **2**, 811.
HADEN, H. C. (1944). *Amer. J. Ophthal.*, **27**, 1.
——— (1947). *Ibid.*, **30**, 1205.
HALLER, A. VON (1754). " Arteriarum oculi historia et tabulae arteriarum oculi ". Göttingen.
HAMILTON, W. J., BOYD, J. D., and MOSSMAN, H. W. (1945). " Human Embryology ". Heffer, Cambridge.
HOLM, E. (1923). *Acta ophthal. (Kbh).*, **1**, 66.
HUSCHKE, E. (1844). "Eingeweide und Sinnesorgane". See Sömmerring (1844).
HYRTL, H. (1839). *Öst. med. Jb.*, **19**, 1.
IGERSHEIMER, J. (1942). *Ophthalmologica (Basel)*, **103**, 230.
JAEGER, E. VON (1861). " Ueber die Einstellungen des dioptrischen Apparates im menschlichen Auge ", pp. 52-56. Vienna; Masson, Paris.
JAYLE, G. E. (1939). " Traite d'ophtalmologie ", vol. 1, p. 347, Masson, Paris.
KEENEY, A. (1951). " Chronology of Ophthalmic Development ". American Lecturer Series. Thomas, Springfield, Ill.
KERSHNER, C. M. (1943). " Blood Supply of the Visual Pathway ". Meador Publ. Co., Boston.
KISS, F. (1943). *Ophthalmologica (Basel)*, **106**, 225.
KRAYENBÜHL, H., and RICHTER, R. (1952). " Die zerebrale Angiographie ". Thieme, Stuttgart.
KUHNT, H. (1879). *v. Graefes Arch. Ophthal.*, **25**, pt. 3, 179.
LEBER, T. (1865a). " Anatomische Untersuchungen ueber die Blutgefasse des menschlichen Auges ", Denkschriften der Wiener Wissenschaft Akademie (Math.-Naturwiss. classe), **24**, 297.
——— (1865b). *Ibid.*, **11**, pt. 1, 1.
——— (1872). *Ibid.*, **18**, pt. 2, 25.
——— (1903). " Die Cirkulations- und Ernährungsverhältnisse des Auges ", *In* " Graefe-Saemischer Handbuch der Augenheilkunde ", vol. 2, p. 1.
LHERMITTE, J. (1939). " Traité d'ophtalmologie ", vol. 1, p. 617. Masson, Paris.
LIEB, E. (1940). *J. techn. Meth.*, **20**, 48.
LOEWENSTEIN, A., and GARROW, A. (1945). *Amer. J. Ophthal.*, **28**, 840.
MAGITOT, A. (1908). " La vascularisation du neuf optique et du chiasma ". Thesè de Paris.
——— (1947). *Ann. Oculist. (Paris)*, **180**, 31.
MANN, I. (1949). " The Development of the Human Eye ", 2nd ed. British Medical Association, London.
MONRO, T. K. (1895). *J. Anat. (Lond.)*, **30**, 45.
RISTITCH, K. (1924). " La vascularisation du nerf optique et du chiasma ". Thesè de Bordeaux.
ROCHON-DUVIGNEAUD. A. (1934). " Recherches sur l'oeil et la vision chez les vertébrés ". Barnéoud, Laval.
SAUTTER, H., and SEITZ, R. (1952). *v. Graefes Arch. Ophthal.*, **152**, 413.
SCHWALBE, G. (1887). " Lehrbuch der Anatomie der Sinnesorgane ". Besold, Erlangen.
SÖMMERRING, S. T. (1844). " Vom Baue des menschlichen Körpers ", vol. 5, " Lehre von den Eingeweiden und Sinnesorganen ", ed. E. Huschke. Leipzig.
SONDERMANN, R. (1948). *v. Graefes Arch. Ophthal.*, **148**, 331.
SUDAKEVITCH, T. (1947). *British Journal of Ophthalmology*, **31**, 738.
TICHOMIROFF, M. A. (1900). " Variations of the Arteries and Veins of the Human Body ". Kiev.
TIEDEMANN, F. (1824). *Z. Physiol.*, **1**, 237.
TRAQUAIR, H. M. (1946). " An Introduction to Clinical Perimetry ", 5th ed. pp. 296–299. Kimpton, London.
TRUETA, J., BARCLAY, A. E., DANIEL, P. M., FRANKLIN, K. J., and PRICHARD, M. M. L. (1947). " Studies of the Renal Circulation ". Blackwell, Oxford.
VAIL, D. (1948). *Amer. J. Ophthal.*, **31**, 1.
VOSSIUS, A. (1883). *v. Graefes Arch. Ophthal.*, **29**, pt. 4, 119.
WALSH, F. B. (1947). " Clinical Neuro-Ophthalmology ", pp. 8–28. Williams and Wilkins Co., Baltimore.
WILCZEK, M. (1947). *British Journal of Ophthalmology*, **31**, 551.
WOLFF, E. (1939). *Trans. ophthal. Soc., U.K.*, **59**, 157.
——— (1940). *Ibid.*, **60**, 69.
——— (1948). " The Anatomy of the Eye and Orbit ", 3rd ed. Lewis, London.
WOLFRING, K. (1872). *v. Graefes Arch. Ophthal.*, **18**, 10.
ZINN, J. G. (1755). " Descriptio anatomica oculi humani ". Wriesberg, Göttingen.

Brit. J. Ophthal. (1954), **38**, 489.

EXPERIMENTAL TOXOPLASMOSIS OF THE UVEAL TRACT*

BY

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Introduction

THE histopathological studies of Wilder (1952) on eyes removed on account of intractable iritis of unknown origin suggest that toxoplasmosis may have been the cause in some cases. Following on her work, requests were received for the serological investigation of patients suffering from chronic anterior uveitis of undetermined aetiology. Some of these gave weak positive results in the low titre ranges, but were of doubtful significance since Beverley and Beattie (1952) found, in a survey of normal people, that 74 per cent. were negative or <1 : 4, 23 per cent. positive with a titre from 1 : 4 to <1 : 16, 2 per cent. with a titre from 1 : 16 to <1 : 32, 1 per cent. with a titre from 1 : 32 to <1 : 64, and none with a titre of 1 : 64 or over. In three patients, however, the levels were high: Case 1, 1 : 160; Case 2, 1 : 64; Case 3, 1 : 96.

In the first case the eye had been removed before the publication of Wilder's work. The eye removed in the second case was unfortunately promptly fixed for histological examination and animal inoculation tests could not be done. Though pseudocysts could not be found in sections of either of these eyes, structures resembling paired vegetative forms could be seen. Unequivocal proof of the latter's identity is not available since recovery of the parasite by inoculation into animals was not attempted, and at the present time there is no staining procedure which is specific for toxoplasms. In the third case the eye was not removed as the condition slowly improved. The possibility of treatment by desensitization on the lines suggested by Frenkel (1949) was discussed and finally rejected. In view of the possibility of recurrence of the uveitis in this case, and bearing in mind future cases, a series of therapeutic experiments were undertaken, the first group of which is now reported.

Experimental Investigations

Production of Toxoplasma Infection of Rabbit's Eye.—Rabbits were anæsthetized with nembutal and open ether. The anterior chamber of the left eye was inoculated with 0·05 ml. of a dilution of peritoneal exudate taken from a mouse inoculated 3 days earlier with the *Rh* strain of toxoplasma. Inoculations were made with a tuberculin type syringe fitted with a Schick test needle which was introduced gently by rotation through the cornea just in front of the limbus and parallel with the

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plane of the iris. The needle was inserted so that its point was at the opposite sector of the anterior chamber before slowly injecting the required dose. This resulted in little of the inoculum escaping when the needle was withdrawn.

Choice of Chemotherapeutic Agents.—In experiments on the treatment of systemic toxoplasmosis in mice and rabbits by chemotherapeutic agents the most encouraging results were obtained from the use of 4: 4′-diaminodiphenylsulphone (hereafter called D.D.S.). This was earlier used by Summers (1949), and two of its soluble derivatives, Diasone and Promin, were used by Cross (1951), in systemic toxoplasmosis of mice produced by intra peritoneal inoculation.

In our experience better results were obtained with D.D.S., which is relatively insoluble, than with four of its more soluble derivatives—Promin, Sulphetrone, Diasone, and 2196 (4 : 4′-bis (ethylamino) diphenylsulphone-α: α′-disodium sulphonate).

Estimation of D.D.S. in Blood and Eye Fluids.—The concentration of D.D.S. in the blood and eye fluids of the rabbits was determined by the colorimetric procedure of Francis and Spinks (1950), in which the Sulphone is coupled to N-β-sulphatoethyl-*m*-toluidine to yield a pink-coloured derivative. The intensity of the latter was measured by means of a Hilger Spekker absorptiometer with green filters (Ilford No. 604), and in the case of the eye fluids micro-cells were used. For the eye fluids, the procedure was essentially that described by Francis and Spinks except that weighed amounts were first treated with trichloracetic acid and the precipitated protein removed by centrifugation. To 0·5 ml. of the protein-free supernatant was added 0·1 ml. $NaNO_2$ (1 mg./ml.) and, after 5 min., 0·1 ml. *N*-β-sulphatoethyl-*m*-toluidine (1 per cent. w/v). The mixture was allowed to stand for 20 min. and then 0·9 ml. ethanol was added.

Dosage and Body Fluid Levels of D.D.S.—When the rabbits were given daily doses of 100 mg. D.D.S./kg. in a gelatin capsule, the concentration of the drug in the blood followed the same sequence as that described by Francis and Spinks (1950). A peak concentration of the order 10 μg. Sulphone per ml. blood was usually attained within 2 hrs and thereafter soon declined, so that 12 hours after dosing the concentration was about 1 μg./ml. With certain rabbits it was noticed that the peak concentration was not so pronounced and was maintained for a much longer period (about 4 hrs). A concentration of at least 10 μg. D.D.S./ml. blood could be maintained throughout the experimental period by dosing the animals at 8-hrly intervals with capsules containing 100 mg. Sulphone per kg. body weight, and rabbits receiving such treatment for a fortnight showed no signs of ill health.

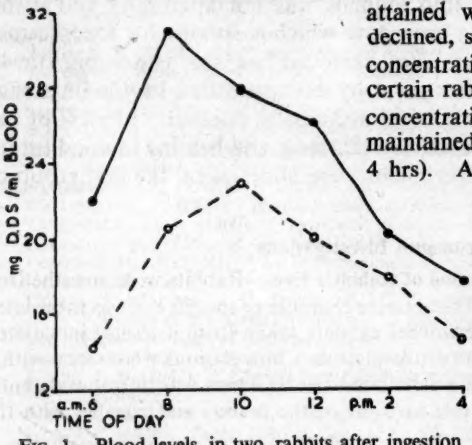


FIG. 1.—Blood levels in two rabbits after ingestion of 100 mg./kg. at 6 a.m., and 8-hrly for the previous 3 days.

Fig. 1 shows the levels of the Sulphone in the blood of two such rabbits during the 10-hr. period

after administration of the last of ten capsules given at 8-hrly intervals over the previous 3 days. The concentration of D.D.S. in the blood and the eye fluids of rabbits being dosed at 8-hrly intervals are recorded in the Table. Rabbits K and J were normal, whilst rabbits IA, IB, II, IV, VA, VB, VI, and VII had been inoculated 3 days earlier with approximately 20,000 toxoplasms into the anterior chamber of the left eye. At the time of taking the samples there was an active unilateral iritis.

TABLE

CONCENTRATION OF 4: 4'-DIAMINODIPHENYLSULPHONE IN THE BLOOD AND EYE FLUIDS OF RABBITS

Rabbit No.	Blood Level (μg./ml.)	Aqueous Humour (μg./g.)			Vitreous Humour (μg./g.)		
		Left	Right	Mean	Left	Right	Mean
IB	51·3	8·0	8·4	8·2	8·5	5·6	7·1
IA	34·5	8·6	9·5	9·1	9·0	9·2	9·1
VA	21·7	8·7	9·5	9·1	—	—	—
II	18·0	5·7	6·5	6·1	7·5	8·3	7·9
K	17·8	7·8	—	—	—	—	—
J	14·6	6·5	—	—	—	—	—
VB	12·3	4·8	4·1	4·5	—	—	—
IV	9·2	4·9	5·1	5·1	5·9	4·1	5·0

Rabbits received doses of D.D.S. at 8-hrly intervals. Blood sample taken immediately before anaesthetizing the animal for withdrawal of sample of aqueous humour, or before death when both aqueous and vitreous humour were required.

Since the eye fluids contain only about 0·3 per cent. solids per 100 g. water, it can be assumed that 1 g. of each of the eye fluids occupies 1 ml., and thus that the concentration of D.D.S. in the aqueous humour is between one-sixth and one-half of that in the blood. It is of interest to note that Sorsby (1949) has recorded that, in rabbits given sulphonamides intravenously, the levels of the drug in the aqueous humour were about 50–60 per cent. of those in the plasma. Though the figures for the concentration of the Sulphone in the vitreous and aqueous humour appear to show some variation between the right and left eye, this is no doubt due to experimental error; from the limited data available it would seem that the concentration of the drug in the vitreous humour is the same as that in the aqueous humour, and that there is no significant difference between the levels of the drug in the inflamed (left) and normal (right) eyes.

Preliminary Experiments

The injection of approximately 750 toxoplasms from a 3-day mouse peritoneal exudate into the anterior chamber of a non-immune rabbit resulted in an acute iritis, usually within 72 to 96 hrs. When the number of organisms was increased to 20,000, the delay in onset of signs of infection was only 30 to 40 hrs. Death in both cases occurred on the 8th or 9th day. If treatment with D.D.S. was begun within 4 to 6 hrs of inoculation with the smaller number of organisms, a good functional result could be obtained, but the animal still developed a severe iritis; with the larger inoculum the animal survived, though usually with a useless eye.

If treatment was withheld until the onset of signs of iritis, a useless eye was nearly always the result, but the animal survived; if treatment was delayed until the iritis was well established, the animal developed a panophthalmitis and died between the 8th and 12th day.

Since in these early experiments the results obtained by the use of D.D.S. alone were not satisfactory, it was decided to try the effect of combining D.D.S. with cortisone on the lines of the experiments of Woods and Wood (1952) on tuberculous iritis. Pilot experiments showed that 10 mg. cortisone given daily by subcutaneous injection produced beneficial effects, and this was the dosage used in systemic treatments in the following experiments.

Therapeutic Experiments

(1) *Effect of D.D.S. and Cortisone.*—Nine pairs of non-immune rabbits were inoculated with approximately 20,000 toxoplasms into the anterior chamber of the left eye by the method described: all inoculations were completed within 1 hr. One pair had no treatment, one pair was treated with cortisone alone, two pairs with D.D.S. alone, and six pairs with a combination of both drugs. Some of the pairs were used to study the effects of delaying treatment for varying periods after inoculation.

All eyes were examined and recorded twice daily in the early days after inoculation and later once daily. After the conclusion of the experiment the severity of the iritis at each examination was assessed on a points system with the following allocation of points:

Conjunctival injection	Mild ½	Moderate 1	Marked 1½
Pupil contraction	Mild ½	Moderate 1	Marked 1½
Colour difference of iris	Slight ½	Definite ½	Marked 1
Pattern of iris	Alteration ½	Loss of detail 1	
Exudate on iris	Flecks ½	Clumps ½–1	Woolly aggregates 1½
Intra-ocular haemorrhage	1		
Chemosis	½–1		
Turbidity of anterior chamber	½–1		
Keratitis	½–1		

The graphs in Fig. 2 (*a-c*) were constructed on this basis and show the effects of the various treatments on the progress or amelioration of the iritis. Because the condition of the eyes in each pair of rabbits ran a very similar course, the graphs were constructed by using the means of the points value for the two animals.

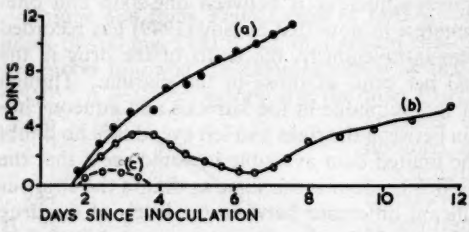


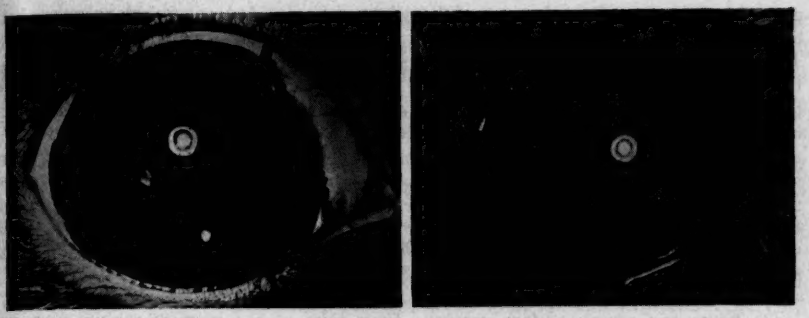
FIG. 2.—Course of iritis
(*a*) In rabbits receiving no treatment.
(*b*) In rabbits treated with D.D.S. (no delay).
(*c*) In rabbits treated with D.D.S. and cortisone (no delay).

After 30-40 hrs the untreated pair (Fig. 2*a*) developed an iritis which progressed steadily till the animals died on the 8th and 9th days (Fig. 3).

The pair treated with cortisone only, starting at the time of inoculation, showed very little difference from the untreated pair. They had a slightly milder reaction in the early stages, but eventually developed an equally severe iritis and died at the same time (8th and 9th days).

The pair treated with D.D.S. from 5 hrs after inoculation (Fig. 2*b*), and the pair whose treatment with D.D.S. was delayed until the first signs of iritis were appearing, *i.e.* 53 hrs (Fig. 4), both had an initial iritis which was followed by a partial remission and then a relapse. The two phases of intense iritis (subsequently referred to as a biphasic response) were more obviously separated in the first of these two pairs.

The pair receiving D.D.S. and cortisone from the start (Figs 2*c* and 5), and the pair receiving D.D.S. from the start and cortisone from the onset of iritis, both developed an iritis of only one phase which corresponded in time with the first phase of the two groups treated with D.D.S. alone. The duration and severity of the iritis were greater in the second pair.



(*a*) Control eye. (*b*) Test eye on 8th day after inoculation.

FIG. 3.—Rabbit receiving no treatment

(*a*) Control eye. (*b*) Test eye on 8th day after inoculation.

FIG. 4.—Rabbit receiving treatment with D.D.S. (48 hrs delay).

(*a*) Control eye. (*b*) Test eye on 9th day after inoculation.

FIG. 5.—Rabbit receiving treatment with D.D.S. and cortisone.

The last three pairs all received D.D.S. from the onset of the signs of iritis (53 hrs), and in addition one pair received cortisone from the outset, another cortisone from the onset of iritis, and the third cortisone at 96 hrs when the iritis was judged to have become well established. All of these, with one exception, had a monophasic response; they showed

general improvement from the peak of the primary phase and were left with eyes showing little damage and good functional result. The exception was one of the eighth pair. This animal developed a generalized bacterial infection with panophthalmitis. Bacteria, but no toxoplasms, were found in the eye after death. The only permanent sequel in the animals treated with both D.D.S. and cortisone was depigmentation of the iris, the intensity of which was proportional to the length of the delay in administering cortisone.

Four of the rabbits receiving cortisone together with D.D.S. died at intervals after the conclusion of the experiment. One died of a generalized bacterial infection 30 days after inoculation and the eye remained quiet throughout its illness. The other three died from acute generalized toxoplasmosis at 19, 17 and 35 days after inoculation, and there was a recurrence of iritis which preceded the death of the animal by 4, 5 and 7 days respectively.

(2) *Route of Cortisone Administration.*—Four pairs of non-immune rabbits were inoculated with 20,000 toxoplasms into the anterior chamber and all were treated with D.D.S. from the 5th hr. The first pair had no other treatment; the second was given 10 mg. cortisone subcutaneously at the time of inoculation and each day afterwards; the third was given 5 mg. cortisone in 0·2-ml. amounts subconjunctivally at the time of inoculation and again at 73 hrs but not afterwards; the fourth pair received cortisone drops thrice daily for 12 days. The drops were made by mixing 1 volume cortisone acetate suspension (25 mg./ml.) with 4 volumes buffered saline diluent containing 4·6 g. NaH_2PO_4, 4·7 g. Na_2HPO_4, and 4·8 g. NaCl per litre of distilled water.

The progress of the pair treated with D.D.S. alone was similar to that of the corresponding pair of the previous experiment, and a biphasic response was again noted. The pair treated with systemic cortisone and the pair treated with subconjunctival cortisone behaved similarly, with only mild inflammatory changes corresponding to the first phase and nothing corresponding to the second phase. The fate of the pair treated with cortisone drops resembled that of the pair treated with D.D.S. alone. The first phase was a little milder, and the onset of the second phase was delayed, but it eventually developed to an even greater intensity.

One of the two rabbits receiving systemic cortisone gradually lost weight, developed weakness of the hind limbs, and was killed on the 19th day after inoculation. One of the two rabbits receiving subconjunctival cortisone remained well for 4 weeks, but then lost its appetite and became thin, and was killed on the 37th day after inoculation. There had been no recurrence of the iritis in either of these animals. The two animals receiving cortisone drops remained fit but developed glaucoma and were killed on the 19th day. The other animals used in this experiment remained well for 63 days.

(3) *Subconjunctival Cortisone.*—To confirm the efficiency of subconjunctival cortisone with D.D.S., three more non-immune rabbits were inoculated with 20,000 toxoplasms into the left anterior chamber. They were given 5 mg. cortisone subconjunctivally at the time of inoculation and 100 mg./kg. D.D.S. 8-hrly. The iritis which followed was generally of a more severe nature than that which occurred in the third pair of Experiment 2, and it was considered necessary to give the subconjunctival cortisone at 48-hr intervals for a total of five doses. All these animals had an iritis of one phase only: the eyes were completely quiescent on and after the 10th day.

Discussion

The biphasic response in the animals treated with D.D.S. alone suggests that two different reactions were involved. The period of least intensity was at the 7th day. We know that serum antibody is detectable on the 10th day in rabbits, and from other experiments with toxoplasmic peritonitis in mice

we have grounds for believing that antibody is produced at the primary site of infection and is detectable there one or two days before it is detectable in the blood serum. We tentatively suggest that the first phase of intense iritis is due to the toxic effect of the organisms and that the second phase may be " reversed allergy ". In the untreated animal these two phases merge into one another, because it is not until the animal develops an antibody mechanism that the organisms are eliminated and the toxic effect ceases, and it is at this phase that the " reversed allergy " mechanism operates. The premature suspension of the toxic effect brought about by the chemotherapeutic action of D.D.S. allows a gap to appear between the two responses, the interval being larger, as would be expected, in the animals receiving earlier treatment. We have noticed similar biphasic responses in the size of skin lesions in rabbits being immunized to toxoplasmosis by intradermal inoculation followed by D.D.S. therapy.

Some support for this theory is provided by the examination of smears of ocular fluids, exudates, and tissues of the animals which died in the preliminary experiments. They showed very few free toxoplasms and those that were seen showed the bloated, faintly staining, reticulated appearance that we associate with antibody-affected organisms. However, " cysts " or terminal colonies were readily seen in sections.

If our surmise is correct, the possibility of reversed allergy in many acute as well as subacute and chronic infections opens up a large field of experimental work, and may perhaps require a revision of some of our conceptions of inflammatory responses.

Cortisone, when given from the start, would also appear to have some effect in reducing the primary toxic effect. Why it should do so is not clear, but there are three possible explanations. It may facilitate passage of D.D.S. into parasitized cells, or it may prevent the interaction of non-specific antibody with toxoplasma antigens, or it may prevent the products of interaction of non-specific antibody with toxoplasma antigens from acting on tissue cells and thus lessening vasodilations, the formation of inflammatory exudates, and the dissemination of parasites.

It is possible that in three of the cortisone-treated animals relapses may have been prevented by a longer course of D.D.S. therapy to cover the period of retardation of antibody formation and/or to eradicate more completely the toxoplasms from intracellular and possibly terminal colony sites. We feel that a further course of treatment with D.D.S. at the onset of the recurrence of iritis would have prevented the death of the animals. Whether a further period of either systemic or local cortisone should have been given as well is a matter for speculation.

The success obtained with subconjunctival cortisone together with the absence of systemic infections and recurrences of the iritis would suggest that this method of administration is superior to the subcutaneous route in early and in mild cases.

Summary

4: 4′-diaminodiphenylsulphone (D.D.S.) proved of value in the treatment of experimentally produced toxoplasmic infection in the anterior chamber of the eyes of rabbits. It was not completely successful, in as much as, though it caused a regression of the primary iritis, a secondary iritis developed after a further 2 or 3 days. It is suggested that the effect of the D.D.S. was to kill the toxoplasms (or most of them) and thus to cut short the primary toxic effects. It did not abolish the antigenic stimulus which after about 7 days resulted in the production of antibodies and a reversed allergy which was responsible for the secondary iritis.

Cortisone, although ineffective by itself, seemed to act by abolishing the secondary allergic iritis, and hence complete, or nearly complete, cures were obtained by the use of a combination of D.D.S. and cortisone. Best results were obtained when the drugs were given within a few hours of infection, but good results were obtained when they were withheld for as long as 53 hrs.

It is suggested that this treatment might be tried in toxoplasmic infection of the eyes in man. It is unlikely that it could be started so soon, but it is also unlikely that so many parasites would be involved in a natural infection.

Our grateful thanks are due to clinical colleagues who sent us the sera from cases of uveitis, and who made many helpful suggestions and criticisms, to the staff of the Photographic Department of the United Sheffield Hospitals for their help and patient perseverance in taking the photographs, to Imperial Chemical Industries for supplies of D.D.S. and other drugs, and to the Medical Research Council for supplies of cortisone.

The work was assisted by a grant to one of us (C.P.B.) from the Medical Research Council.

REFERENCES

BEVERLEY, J. K. A., and BEATTIE, C. P. (1952). *J. clin. Path.*, **5**, 350.
CROSS, J. B. (1951). *Proc. Soc. exp. Biol. (N.Y.).*, **76**, 548.
FRANCIS, J., and SPINKS, A. (1950). *Brit. J. Pharmacol.*, **5**, 565.
FRENKEL, J. K. (1949). *J. Amer. med. Ass.*, **140**, 369.
SORSBY, A. (1949). *British Journal of Ophthalmology*, **33**, 347.
SUMMERS, W. A. (1949). *Amer. J. trop. Med.*, **29**, 889.
WILDER, H. C. (1952). *Arch. Ophthal. (Chicago)*, **48**, 127.
WOODS, A. C., and WOOD, R. M. (1952). *Ibid.*, **47**, 477.

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EXTRACONJUNCTIVAL APPROACH TO THE EXTRA-OCULAR MUSCLES*

BY

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SINCE contact lenses have been used for monocular conditions of impaired vision in which the eye is divergent, *e.g.*, aphakia and corneal scars, it has been found that although vision could be improved the affected eye remained divergent.

Some of these eyes, *i.e.* those with small degrees of divergence, attained single binocular vision with orthoptic treatment; others with larger angles had to be helped surgically.

Orthodox trans-conjunctival surgery on these muscles was not very suitable for the following reasons:

(*i*) The majority of lenses which can be worn with comfort and without corneal haze require large haptic surfaces and need to be ventilated.

(*ii*) The haptic surfaces of these lenses extend beyond the sites of insertion of the muscles, and any scarring of this area naturally upsets the fit and balance of the lenses, rendering them unwearable.

(*iii*) In certain cases, although the lens might still be wearable, the haze-free time is shortened.

(*iv*) In yet other cases, the ventilating bubble increases in size owing to the disturbance in the fit, thus interfering with clear sight.

To overcome these difficulties an attempt was made to reduce the diameter of the lenses, but this was found to upset their balance. The contact lens in these cases has to be fitted before any operative intervention in order to obtain the full benefit of restored visual acuity and orthoptic treatment; the residual divergence only can then be treated surgically. The orthodox transconjunctival approach to the muscles in these cases would therefore mean scarring in the haptic area of the lens with the consequences described above. Refitting over a scarred area is the only alternative, and this is a technically difficult process.

Taking into consideration the various factors, I thought it would be best to approach the muscles away from their insertions to allow the scar tissue to form well away from the haptic area of the lens, and so devised the technique of extraconjunctival approach to the muscles, which has proved uniformly successful so far.

Procedure

Instruments.—As for a routine squint operation with a double-armed 000 catgut suture if not used normally and a Graefe knife.

Anaesthesia.—General.

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Technique.—The lids may be infiltrated with saline and adrenaline with advantage.

(1) External Rectus

(*a*) The lids are held apart by the assistant and the eye turned inwards by a traction suture at the limbus, thus exposing the external canthus. An incision is now made as in Fig. 1 (*a*) with a view to separating the conjunctiva from the outer ends of the upper and lower tarsal plates.

(*b*) This separation is continued with fine scissors backwards and towards the globe. This dissection exposes a mass of tissue consisting of the lateral check ligament, connective tissue, and the sheath of the external rectus muscle (Fig. 1*b*).

(*c*) The external check ligament is now cut (Fig. 1*c*).

(*d*) An incision is now made in the connective tissue and sheath in the direction of the muscle fibres. The connective tissue and sheath are reflected upwards and downwards and the muscle exposed. This procedure is necessary to avoid any chance of injury to the nerve to the inferior oblique which runs along the inferior border of the lateral rectus part of the way (Fig. 1*d*).

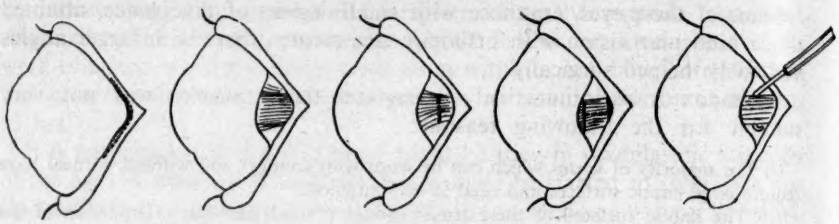
(a) Line of incision
(b) Reflected conjunctiva
(c) Line of incision through check ligament of external rectus
(d) Incision along length of muscle
(e) Muscle on hook

FIG. 1.—External rectus muscle approach.

(*e*) The muscle is now picked up on a squint hook (Fig. 1*e*); its identity may be confirmed by pulling it laterally, when the eye should turn in the direction of action of the muscle and a small dimple should appear at the site of insertion of the muscle. It is necessary to be quite certain of the identity of the muscle as the inferior oblique is inserted in this region and may be picked up in error.

(*f*) If a resection is required it is carried out in the usual way. As a recession is not possible by this technique the muscle has to be lengthened. The muscle fibres are cut half-way across and then along for a distance slightly longer than half of the total lengthening required, again as in Fig. 2*a*. The two cut ends are then sutured together by means of a mattress suture as shown in Fig. 2*b*. The muscle is now replaced, the wound closed and the tissues allowed to fall back into position. Only one stitch is inserted and then across through the conjunctiva and the apex of the external canthus. No dressing is required.

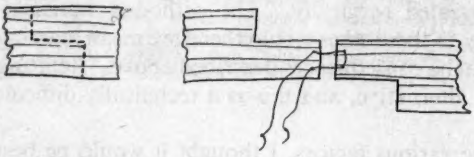

FIG. 2.—Lengthening of muscle.

(2) Internal Rectus

(*a*) The lids are held apart by means of a speculum and the eye turned outwards by a suture at the limbus. An incision is made along the lateral margin of the caruncle (Fig. 3*a*).

(*b*) The conjunctiva is dissected outwards from the skin of the caruncle. This dissection exposes the internal rectus muscle covered by its check ligament and sheath and surrounded by connective tissue (Fig. 3*b*).

(*c*) The muscle is picked up on a squint hook and cleared of its sheath and tissue and its identity confirmed as for the external rectus (Figs 3*c* and 3*d*).

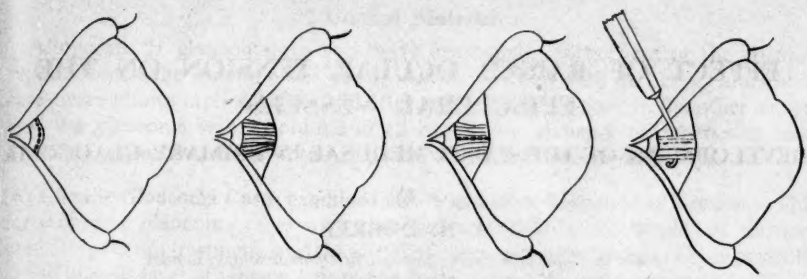

FIG. 3.—Internal rectus muscle approach.

(*d*) The necessary resection or lengthening is carried out.
(*e*) The muscle is replaced, haemostasis achieved and the tissues smoothed back into position.
(*f*) The conjunctiva is stitched back to the caruncle with one suture. No dressing is required.

Motility exercises should be started the next day, and the contact lens can be worn. Occasionally there is some conjunctival chemosis, usually caused by indifferent haemostasis at the end of the operation or by reaction to the catgut. This would naturally interfere with lens wearing, but can be controlled rapidly by using gutt. argyrol 5 per cent. with adrenaline. Motility exercises should be carried out whilst the eye is still oedematous, as they help to reduce the oedema. Orthoptic treatment should commence as soon as the lens can be worn.

For small divergent angles only a resection of the internal rectus is needed. A 4-mm. resection secures a correction of about 5–8°. For larger angles the lateral rectus has to be tackled as well—a 4-mm. resection of the internal rectus and an 8-mm. lengthening of the external rectus can correct an angle of 25–30°.

Discussion

As stated above this technique has been uniformly successful so far, and it was therefore thought advisable to compare it with the orthodox technique of extra-ocular surgery. An experiment was carried out on two cases through the courtesy of Mr. Hatfield Wright of Swindon. Bilateral muscle surgery had been decided on in these two cases, and at operation Mr. Wright did one eye using the orthodox technique whilst I did the other one using the extra-conjunctival approach. No difference in time of healing, extent of mobility of the eye, or post-operative oedema or comfort of the children was observed. Apart from the site of the scar the result was identical. As the extra-conjunctival method does not appear to offer any advantage over the trans-conjunctival technique, and is certainly more difficult to carry out, I think that it should be used only in contact lens cases or special circumstances of like nature.

I wish to record my appreciation of Mr. Hatfield Wright's generosity in providing patients and hospital facilities for the experiment. My thanks are due to my technician Geoffrey Rhodes for the drawings.

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EFFECT OF RAISED OCULAR TENSION ON THE EPISCLERAL VESSELS*

DEVELOPMENT OF THE CAPUT MEDUSAE IN PRIMARY GLAUCOMA

BY

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SINCE Mackenzie (1854) published his classical descriptions of glaucoma, his " varicosity of the external vessels " has been recognized as an important physical sign of the later stages of the disease. There is still, however, no general concensus of opinion as to how, or why, the vascular changes develop.

The results of an investigation into the vascular changes occurring in the transient *diurnal* variations of pressure of chronic glaucoma have recently been presented. Observations on the episcleral vessels were made by serial photographs, and among the conclusions were two of importance to the present study. The first was that, if the tension of the eye observed was moderate or low in degree, a physiological vaso-dilatation, mainly affecting the venous side of the circulation, was associated with the periods of *lowered* tension. The second was that, if the tension rose to sufficient heights, a dilatation of the anterior ciliary arteries occurred (Dobree, 1953). The latter finding was thought to be secondary to the pressure itself and this view was further supported by the observation, subsequent to successful filtering operations, that these same vessels showed a marked decrease in calibre.

This suggested one method of studying the relationship between the ocular pressure and the associated vascular changes occurring at the higher tension levels. For, by photographing the vessels before and after operation in a large series of cases in which the general level of tension was known, information would be obtained regarding the degree of tension necessary to produce the vascular changes. Another method which presented itself was to study a series of cases in which the reduction of tension had been effected by miotics. Finally, in order to observe the extreme type of case in which the pressure had been very high and prolonged, a number of selected cases of absolute primary glaucoma were studied.

Technique

A 35-mm. camera was fitted to one side of a slit-lamp binocular in place of the eye-piece, and visual observations were made through the second eye-piece. The termination of a suitable anterior ciliary artery, preferably with a large vein lying alongside, was selected. Preliminary focussing was done with the narrow beam of the slit-lamp and when the required area was in focus the slit was opened to its full extent and the shutter released. After the photograph was taken, the tension was taken by a Schiötz tonometer.

*Received for publication March 5, 1954.

Clinical Material

Altogether 51 glaucomatous eyes were examined; forty were of the chronic type in which the function was good, the vision being 6/18 or better, and these cases were photographed before and after reduction of tension; in the other eleven eyes the glaucoma was absolute and all had vision of hand movements or less. The changes in these cases were compared with a low tension fellow eye.

(A) Chronic Glaucoma Cases examined before and after Reduction of Tension.—The forty chronic glaucoma cases were classified according to the height of tension prevailing before trestment, and the initial photographs were taken as far as possible at the highest level of tension before any form of therapy had been started. They were grouped according to the initial tension as follows:

Above 60 mm. Hg	... ...	High tension (eleven cases)
Between 30–60 mm. Hg	... ...	Moderate tension (twenty-one cases)
Below 30 mm. Hg	... ...	Low tension (eight cases)

This grouping is approximate as the diurnal variations are sometimes considerable, and, although all cases were phased for at least 6 hours at a subsequent examination, it is possible that some of these classified as of moderate tension should have occasionally qualified for inclusion in the high-tension group. However, as the series is a large one, and as the object was to determine the general pattern of vascular change, it is probable that there is no great error in adopting this rather over-simplified method of classification.

(*i*) the eleven high-tension cases included six of congestive and five of chronic simple glaucoma.

(*ii*) The 21 moderate-tension cases included six congestive, thirteen chronic simple, and two juvenile glaucomas.

(*iii*) The eight low-tension cases comprised two congestive and six chronic simple glaucomas.

The forty eyes also fell into two series according to the treatment adopted:

Series 1, photographed before and some 2 months after reduction of tension by operation, included six high, eleven moderate, and two low-tension cases.

Series 2, photographed before and after control by miotics, consisted of five high, ten moderate, and six low-tension cases.

The object of studying this second series was two-fold: first the greater control available in the reduction of tension obtained by miotics proved a convenient means of finding out how long it took the changes in calibre to regress; secondly, it was important to ensure that the vascular changes following filtering operations were not due to the surgical procedures themselves, but were directly related to the reduction in tension. As an additional precaution, to ensure that the changes in calibre were not consequent on the use of miotics themselves, some observations were carried out on ten normal and low-tension eyes to ascertain the normal response of the episcleral vessels to eserine and pilocarpine. A more detailed account of these and other findings will be published subsequently, but the main effects of these drugs as shown by serial photographs, are as follows:

Eserine, in 0·5 or 1 per cent. solution, usually causes an immediate spasm of both the episcleral arteries and veins, but after 10–15 minutes these again dilate. This dilatation is much more in evidence on the venous side of the circulation than on the arterial, and lasts for several hours.

Pilocarpine, in 1 or 2 per cent. solution, has a pure dilatatory effect, again mainly on the veins and capillaries, but more transient.

These tests showed that the miotics themselves were not responsible for the decrease in the calibre of the arteries which occurred when the ocular pressure was reduced by their agency.

(B) Absolute Glaucoma Cases.—Although absolute glaucoma is still a relatively common condition and there are often gross changes in the calibre of the episcleral vessels in most of the examples seen, the changes could be related to the causal lesion rather than to the raised tension itself. Cases of absolute thrombotic glaucoma, for example, usually show prolific new vessel formation on the iris and in the filtration angle. Again, cases of absolute congestive glaucoma often have antecedent attacks in which ciliary congestion is a prominent feature. Considerable care was therefore taken to exclude any case of secondary glaucoma or of primary glaucoma with a history of acute attack. Furthermore, as a direct comparison was made between the two eyes, it was necessary that in this series the fellow eye showed, at the most, only a moderate degree of tension. Out of some 1,000 available glaucoma patients, only eleven had eyes which answered these somewhat exacting requirements, and they were of the following types:

Absolute simple glaucoma	7
Absolute congestive glaucoma (without antecedent acute attack) ...	3
Absolute glaucoma capsulare	1

Findings

(A) Chronic Glaucoma Cases examined before and after Reduction of Tension

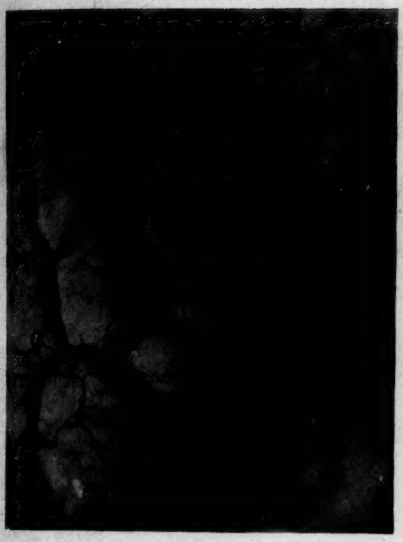
(1) *Changes in Calibre of Anterior Ciliary Arteries.*—There were eleven high-tension cases, of which six had the pressure reduced by operation and five by miotics. All except one showed a marked reduction in the calibre of the arteries when the tension had been lowered (Figs 1*a* and 1*b*). In five cases this decrease in calibre amounted to about one-half of the original diameter, and in the others to between one-third and one-quarter.

Of the 21 moderate-tension cases, eleven had the tension reduced by operation and ten by miotics. Six of the cases operated upon and three of the others showed a definite reduction in arterial calibre, although the reduction was less marked in most instances than in the high-tension group. Only one with initial tension lower than 40 mm. Hg showed this decrease, and that was a juvenile glaucoma case with an initial tension of 35 mm. Hg.

No changes were noted in any of the eight low-tension cases; These comprised two in which the pressure was lowered by operation and six in which this was effected by miotics.

The actual branches of the arteries affected by the changes in calibre showed some interesting points. They did not show an equal degree of dilatation; the main perforating stems, for example, usually showed more alteration than their smaller superficial branches which, indeed, were often unchanged after the operation.

It was shown by Leber (1873), and recently by Ashton and Smith (1953),

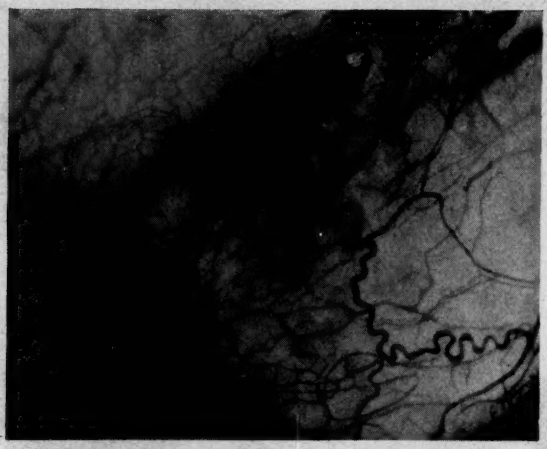


(*a*) Untreated case at initial examination, tension 80 mm. Hg Schiötz.

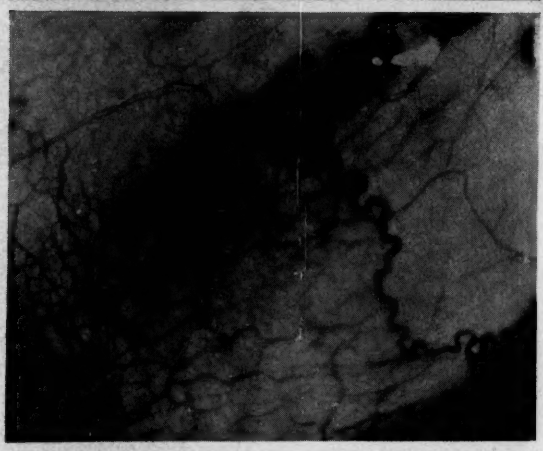
(*b*) The same 2 months after operation, tension 25 mm. Hg Schiötz.

FIGS 1 (*a*) and 1 (*b*).—Calibre change of anterior ciliary artery in chronic simple glaucoma after reduction of tension by operation.

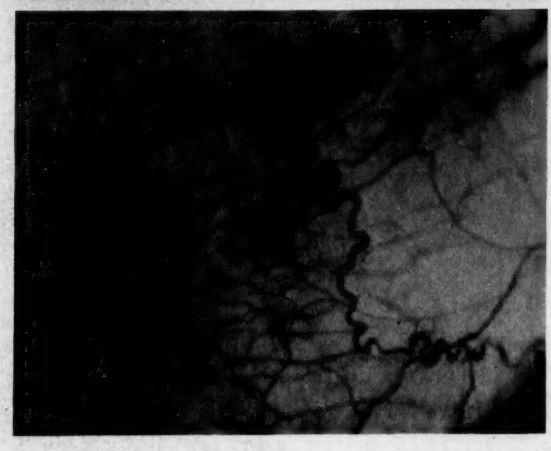
A=anterior ciliary artery,
B=vein arising from vicinity of deep scleral plexus,
C=vein arising from superficial episcleral venules.



(*a*) Untreated chronic simple glaucoma, tension 63 mm. Hg Schiötz.



(*b*) 2½ hours after initial instillation of pilocarpine, tension 22 mm. Hg Schiötz.



(*c*) 2 weeks after continuous pilocarpine therapy, tension 22 mm. Hg Schiötz.

FIGS 2 (*a*) to 2 (*c*).—Fall in tension not immediately followed by reduction in arterial calibre.

that these main arterial trunks are destined for the interior of the globe, and that the smaller branches are superficial and destined mainly for the deep scleral plexus in the region of the canal of Schlemm.

(2) *Time Relationships of Calibre Changes.*—A striking fact which emerged from a review of the cases in which the pressure was reduced by miotics was that a reduction in arterial calibre did not immediately follow the reduction in tension, so that arteries did not attain their fully-constricted state for several days (Figs 2*a* to 2*c*). This may explain the constancy in arterial calibre in the diurnal tension variations of the cases of moderate tension to which attention was previously drawn (Dobree, 1953).

(3) *Changes in Episcleral Veins.*—The trunks of the main episcleral veins were, if possible, included in the photographs. In contrast to the changes on the arterial side of the circulation, there was very little alteration in the calibre of the venules and veins before and after operation. This was true whether the vein derived from superficial tributaries or emerged as a sizeable trunk, presumably from the deep scleral plexus. In Figs 1*a* and 1*b*, for example, although the calibre of the artery diminished to less than one-third its original size, both the deep and superficial episcleral veins showed very little change in calibre. It has been previously shown that after filtering operations the diurnal fluctuations in the calibre of the veins continue as before, and it was therefore important to photograph the vessels at comparable times of the day. Further, as might be expected from the observations on the effect of miotics on the episcleral vessels described above, in those cases wherein the tension was reduced by this means, there was some dilatation of the veins, but the alterations in calibre were not as striking as those occurring in the arteries.

It must be noted here that this discrepancy between the calibres of the arteries and of the veins is a most unusual finding. In ciliary congestion, for example, however caused and however mild, an arterial dilatation comparable to that just described is associated with an enormous dilatation of the venules and veins and a great increase in the capillary network. It must be emphasized that in these cases of chronic glaucoma the eyes were quite white at the height of tension, and were not the subject of anything in the nature of a sub-acute attack.

(B) Changes in Cases of Absolute Glaucoma

In these cases a direct slit-lamp comparison was made between the two eyes and, as has been stated, the cases were so selected that it was possible to compare the vessels of the high-tension eye with those of a low-tension fellow eye.

All of the eleven eyes had a tension of 60 mm. Hg or over. In one case, with a tension of 63 mm. Hg, there was little difference between the vessels of the two eyes, but in the remainder the differences were striking. In the first place the average diameter of the anterior ciliary arteries was double

that in the fellow (low-tension) eye. Furthermore, another important difference became apparent; a dilatation of the veins was also present in some of the more long-standing cases. Thus, three eyes showed an arterial dilatation alone, two showed a definite dilatation of some of the veins in addition, and in the other five there was a gross increase in the diameter of some of the veins as compared with the main trunks in the fellow eye.

The veins in question are large trunks which originate near the limbus. They form in pre-existing channels which are often quite insignificant before the rise in tension. They have important tributaries from the venous ends of the limbal loops or from corneal pannus vessels which are often present in these cases. Other tributaries emerge from the superficial episcleral venous plexus lying adjacent to the limbus and also from branches which presumably derive from the deep corneal plexus as well. The limbal loops, the pannus vessels, and the superficial episcleral veins normally obtain part of their arterial supply from minute twigs which spring from the anterior ciliary arteries before the latter perforate the sclera (Graves, 1934; Dobree, 1950). Now in those cases where the veins were engorged, these superficial twigs were found to be greatly enlarged and were clearly sending more blood to the region of the limbus than in normal eyes. There is therefore an anatomical basis for supposing that where the dilated veins are a prominent feature a quantity of arterial blood is diverted into them through superficial channels. In point of fact, it is probable that much of the arterial supply of the superficial episcleral circulation comes from arterial twigs lying more deeply in the sclera in the region of Schlemm's canal, but these too are purely superficial vessels which derive from the smaller perforating branches of the anterior ciliaries which form a plexus in the vicinity of the canal (Ashton and Smith, 1953).

A further interesting difference between those eyes which showed a purely arterial dilatation and those in which there were also engorged venous trunks concerned the state of the crystalline lens; in the former the lenses were clear, but where the veins were dilated there was a cataract. This may add weight to the suggestion which will be made below, namely, that an arterial dilatation is the initial stage in the formation of a caput medusae, and that where a venous engorgement is also present a much more serious circulatory embarrassment is indicated.

Interpretation of Vascular Changes

It will be recalled that no vascular changes were found in the low-tension cases and that it is unusual to find any unless the tension rises above 40 mm. Hg. Above this level the proportion of cases showing changes in the arteries increases, until at tensions above 60 mm. Hg an arterial dilatation is nearly always present. It is likely, therefore, that the increase in diameter of the anterior ciliary arteries is a direct result of the raised intra-ocular pressure. The anterior ciliary arteries constitute only one group of arteries entering the globe, but the behaviour of the others may well follow the same pattern.

It is suggested that the cycle of events may be as follows: if, for any reason, the ocular pressure rises, this pressure is transmitted in the first place to the intra-ocular veins, and hence the resistance to the flow of blood through the eye is increased. This will tend to cause a reduction in the quantity of blood flowing through the globe in a given time. The resistance to flow will cause the pressure in the arteries to rise, and if there is, at the same time, a widening of their lumina, the rise will be further augmented. In other words, the arterial pressure-head will increase so that the quantity of blood flowing through the eye will, to a greater or lesser extent, be restored.

The dilatation of the arteries may be purely mechanical in origin, analogous to the distension of the pulmonary artery which occurs when the pressure in the pulmonary veins is increased in conditions such as mitral stenosis, emphysema, or, more acutely, in pulmonary embolus. The vessels now under consideration are, however, of a quite different order of magnitude, and are sufficiently small to be under nervous or chemical control; it is thus theoretically possible that their calibre could be altered by changes of this nature occurring within the globe, but the fact that the distension of the arteries takes some time to regress, strengthens the belief that it is mainly mechanical in origin.

The changes noted in the cases of absolute primary glaucoma may be explained as follows:

In cases which show only arterial distension, the circulation may be compensated for the rise in ocular tension by the mechanism already suggested; indeed the absence of cataract in such eyes suggests that the intra-ocular circulation is adequate. Cases in which large venous trunks appear among the episcleral vessels always have an associated cataract, which may indicate a considerable defect in the intra-ocular blood supply. The explanation of the presence of these large veins, which, it will be remembered, arise near the limbus, may be simply that the arterial pressure cannot surmount the ocular pressure in degree sufficient to do more than maintain a greatly reduced circulation inside the eye and that the arterial blood is short-circuited on the surface of the globe *via* the superficial vessels into the limbal loops and superficial and deep scleral plexi.

No positive information regarding the state of the intra-ocular circulation can be deduced from this study, but it is possible that in extreme cases of tension very little blood passes into the interior of the globe and that most of it is shunted through these superficial anastomoses. Further, if this already inadequate intraocular circulation were further reduced, a point might be reached, when the ocular tension would actually fall, a possible explanation of the phthisis bulbi which marks the final stage of glaucomatous degeneration.

Summary

The changes in the episcleral vessels after prolonged periods of raised ocular tension have been investigated.

In chronic glaucoma, of both the chronic simple and congestive types,

the most striking change was a dilatation of the anterior ciliary arteries. This finding was uncommon when the tension was below 40 mm. Hg, but above 60 mm. Hg it was nearly always present. The perforating branches of the arteries were particularly involved, and, after the reduction of the intraocular pressure, the arterial distension took at least some days to regress. The episcleral veins, by contrast, showed little change.

Some advanced cases of absolute primary glaucoma, in which the tension was particularly high and prolonged, showed a number of greatly distended episcleral veins in addition to the arterial dilatation.

The significance of these findings is discussed. The arterial dilatation seems to be a compensatory change consequent on the raised tension, and the occurrence of dilated veins probably implies that much of the blood destined for the interior of the globe has been short-circuited into the superficial veins through anastomotic channels in the vicinity of the limbus.

I should like to express my thanks to Sir Stewart Duke-Elder for his interest and helpful criticism, to Dr. M. Langham, Dr. J. F. Heggie, and Mr. M. Bates for guidance on the physiology and pathology of blood vessels, and to the Medical Illustration Dept. of the Institute of Ophthalmology, for help in the preparation of the photographs.

REFERENCES

ASHTON, N., and SMITH, R. (1953). *British Journal of Ophthalmology*, **37**, 577.
DOBREE, J. H. (1950). *Ibid.*, **34**, 720.
——— (1953). *Ibid.*, **37**, 293.
GRAVES, B. (1934). *Ibid.*, **18**, 305.
LEBER, T. (1873). *V. Graefes Arch. Ophthal.*, **19**, Pt. 2, p. 91.
MACKENZIE, W. (1854). "A Practical Treatise on the Diseases of the Eye", 4th ed. Longman, Brown, Green and Longmans, London.

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HYPERTROPIA CORRECTED BY CHANGES OF THE LATERAL RECTUS INSERTION*

BY

JOHN FOSTER and E. C. PEMBERTON

EIGHT years ago we published 26 cases of concomitant convergent squint (Foster and Pemberton, 1946), which had been treated conventionally by advancement of the lateral rectus and recession of the medial rectus, plus an elevation or depression of either the medial or lateral rectus insertion, to correct a small vertical error. Our results showed that:

(1) elevation of a lateral rectus insertion raised the eye.

(2) depression of a lateral rectus insertion lowered the eye.

(3) cyclophoria occurred in two cases only and was slight.

We concluded that the operation had some justification when a vertical error was constant and less than 11 prism dioptres at all horizontal angles on the synoptophore.

Alvaro (1950) had extended the procedure by raising the insertion of both medial and lateral recti simultaneously in the same eye.

As we still encounter colleagues who not only consider that raising a lateral rectus lowers the eye and are prepared to prove it geometrically, we feel that our first series of cases failed to produce complete conviction and that the publication of a further series, 29 in number, might be timely. The cases recorded in the Table overleaf include all those we have treated in this way since 1948, the relatively small number being due to an increased readiness to operate on the obliques with an improvement in the technique of such operations. It will be observed that:

(1) the new results are " on all fours " with the first series—raising of the external rectus raises the eye;

(2) Alvaro found that raising or lowering of both lateral recti simultaneously, alters the position of the eye in a way consistent with our findings;

(3) cyclophoria is absent in this series.

It is interesting to note that the raising of both lateral recti produces less effect than we had expected, in view of the effect gained from the operation on one muscle.

Although the surgery of the obliques has advanced considerably in Great Britain, we still feel that there is a place for this operation, and our views as to its effect on the position of the eye remain unchanged.

REFERENCES

FOSTER, J., and PEMBERTON, E. C. (1946). *British Journal of Ophthalmology*, **30**, 88.
ALVARO, M. E. (1950). *Ophthalmologica* (*Basel*), **120**, 191.

*Received for publication December 28, 1953.

Serial Case No.	Operation	Pre-operative Measurement (with correction)	Post-operative Measurement (with correction)	Subsequent Measurement (with correction 1–3 mths later)
1	4 mm. recession L.M.R. 7 mm. resection L.L.R. ½-width raising L.L.R.	+15° R/L 6△	FRE −10° L/R 2△ FLE −14° L/R 2△	0° L/R 2△
2	4 mm. recession R.M.R. 5 mm. resection R.L.R. ½-width raising R.L.R.	FRE +15° L/R 8△ FLE +18° L/R 8△	FEE −6° L/R 7△	+ 3° ⊖
3	5 mm. recession L.M.R. 7 mm. resection and 4 mm. advancement L.L.R. ½-width lowering L.L.R.	+31° L/R 7△	− 3° R/L 3△	
4	4 mm. recession R.M.R. ½-width lowering R.M.R. 6 mm. resection R.L.R. ½-width lowering R.L.R.	+17° R/L 8△	+ 6° ⊖	+ 3° L/R 2△
5	4 mm. recession L.M.R. ½-width raising L.M.R. 4 mm. resection L.L.R. ½-width raising L.L.R.	+14° R/L 8△	+12° L/R 4△	Without glasses = − 3° L/R 4△
6	4 mm. recession R.M.R. ½-width raising R.M.R. 8 mm. resection R.L.R. ½-width raising R.L.R.	No glasses worn FRE +28° L/R 15△ FLE +25° L/R 13△	No glasses worn FRE + 7° L/R 15△ FLE + 2° L/R 13△	No glassess worn FEE + 3° L/R 8△
7	5 mm. recession L.M.R. 12 mm. resection L.L.R. 4 mm. raising L.L.R.	+44° R/L 7△	+10° L/R 5△	+ 4° L/R 5△
8	4 mm. recession L.M.R. 7 mm. resection L.L.R. ½-width raising L.L.R.	+19° R/L 6△	+ 6° L/R 6△	
9	5 mm. resection R.M.R. 2 mm. raising R.M.R.	FRE −13° L/R 11△	FEE − 2° L/R 6△	FEE − 5° L/R 3△
10	5 mm. recession L.M.R. 8 mm. resection L.L.R. ½-width lowering L.L.R.	+27° L/R 6△	+20° L/R 6△	+ 5° L/R 1△
11	3 mm. recession L.M.R. ½-width lowering L.M.R. 4 mm. resection L.L.R. ½-width lowering L.L.R.	No glasses worn +18° L/R 9△	No glasses worn − 3° L/R 2△	No glasses worn − 2° R/L 2△
12	4 mm. recession L.M.R. ½-width raising L.M.R. 3 mm. resection L.L.R. ½-width raising L.L.R.	+16° R/L 6△	+ 9° R/L 2△	+ 9° R/L 2△
13	5 mm. recession L.M.R. ½-width raising L.M.R.	+12° R/L 4△	+ 5° R/L 1△	+ 4° ⊖
14	5 mm. recession L.M.R. 4 mm. resection L.L.R. ½-width raising L.L.R.	FRE +18° R/L 4△ FLE +22° R/L 9△	+ 8° L/R 17△	FRE + 9° L/R 13△ FLE +13° L/R 9△

Serial Case No.	Operation	Pre-operative Measurement (with correction)	Post-operative Measurement (with correction)	Subsequent Measurement (with correction 1–3 mths later)
15	resection R.L.R. ½-width raising R.L.R.	+15° L/R 8△	+15° L/R 8△	+ 7° L/R 4△
16	3 snip-tenotomy R.M.R. 4 mm. resection R.L.R. ½-width lowering R.L.R.	FRE + 5° R/L 8△ FLE + 5° R/L 10△	FRE − 8° R/L 3△ FLE − 8° R/L 6△	0° ⊖
17	4 mm. recession R.M.R. 8 mm. resection R.L.R. 3 mm. lowering R.L.R.	FRE +17° R/L 5△ FLE +28° R/L 9△	FEE + 8° L/R 5△	Without glasses FRE − 4° L/R 7△ FLE + 5° L/R 7△
18	4 mm. recession R.M.R. 4 mm. resection R.L.R. ½-width raising R.L.R.	+ 9° L/R 6△	+ 5° ⊖	− 6° ⊖
19	4 mm. recession L.M.R. 5 mm. resection L.L.R. ⅓-width lowering L.L.R.	No glasses worn +16° L/R 3△	No glasses worn + 8° ⊖	No glasses worn + 5° ⊖
20	4 mm. recession L.M.R. 6 mm. resection L.L.R. ½-width raising L.L.R.	+18° R/L 9△	− 2° R/L 2△	+ 4° L/R 3△
21	5 mm. recession R.M.R. 8 mm. resection R.L.R. ½-width lowering R.L.R.	FRE +25° R/L 6△ FLE +28° R/L 13△	+15° R/L 10△	+11° ⊖
22	4 mm. recession L.M.R. ½-width lowering L.M.R. 5 mm. resection L.L.R. ½-width lowering L.L.R.	FRE +21° L/R 7△ FLE +22° R/L 5△	+15° L/R 5△	FRE +17° L/R 3△ FLE +15° ⊖
23	4 mm. recession R.M.R. 3 mm. resection R.L.R. ½-width raising R.L.R.	+ 8° L/R 3△	+ 8° ⊖	+ 8° ⊖
24	5 mm. recession L.M.R. 10 mm. resection L.L.R. ½-width raising L.L.R.	+30° R/L 8△	+ 8° R/L 2△	After further recession R.M.R. own glasses = 0° L/R 3△
25	5 mm. recession R.M.R. 5 mm. resection R.L.R. 6·5 mm. lowering R.L.R.	No glasses worn FRE +30° R/L 13△ FLE +26° R/L 13△	No glasses worn FRE +14° L/R 2△ FLE +14° ⊖	No glasses worn FEE + 2° L/R 1△
26	Recession and lowering L.L.R.	No glasses worn FRE − 8° L/R 6△ FLE − 4° L/R 2△	No glasses worn FEE +1−13° L/R 2△	No glasses worn +1° − − 1° ⊖
27	4 mm. recession L.M.R. 6 mm. resection L.L.R. ½-width lowering L.L.R.	+17° L/R 3△	+ 2° ⊖	− 4° ⊖
28	4 mm. recession R.M.R. whole width raising R.M.R. 8 mm. resection R.L.R. whole width raising R.L.R.	FRE +22° L/R 16△ FLE +22° L/R 12△	FEE 0° R/L 1–2△	
29	5 mm. recession R.M.R. ½-width raising R.M.R. 6 mm. resection R.L.R. ½-width raising R.L.R.	FRE +23° L/R 7△ FLE +23° L/R 4△	FEE +10° ⊖	

KEY TO TABLE

L.M.R.	Left medial rectus	F.R.E.	Fixing right eye
L.L.R.	Left lateral rectus	F.L.E.	Fixing left eye
R.M.R.	Right medial rectus	F.E.E.	Fixing each eye
R.L.R.	Right lateral rectus	⊖	No vertical error

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LOCALIZED INTRA-ORBITAL AMYLOID DISEASE*

BY

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AMYLOID disease or amyloid degeneration used to be met with much more commonly before the present era of medicine, in patients suffering from prolonged toxic states, particularly syphilis, tuberculosis and chronic sepsis. Chronic avitaminosis, mainly deficiency in the vitamin B complex, has been held by many to be a factor in the production of this degeneration.

With the present advance in prevention and treatment of such prolonged toxic states and with the routine administration of vitamins to such patients, this form of degenerative condition has greatly diminished and is now much more rarely seen.

It occurs both in a *generalized* and in a *localized* form. In the generalized form more than one organ is involved, usually together with the liver, spleen, and kidneys, while in the localized form, the disease is limited to one organ and is occasionally given the name " amyloid tumour ". The latter variety is known to occur as a primary process in the tissues of the larynx and also as a secondary degeneration in a pre-existing tumour; it may also occur in areas of chronic inflammation.

Case Report

The following is an instance of amyloid degeneration in an inflammatory mass in the orbit, casuing proptosis.

A man, aged 45, complained of left proptosis of 13 months' duration (Fig. 1). He gave a history of an attack of inflammation of both eyes 3 years previously which left him with blurred vision; 13 months before he was seen by me, he became conscious of a painless mass in the inner part of the left orbit and since then noticed that his left eye was gradually protruding.

During the last 4 years he had had dental sepsis because of which several teeth were extracted, and he was left with a discharging oro-maxillary fistula in the right upper jaw.

He had no history of syphilis, and had not suffered from diplopia.

FIG. 1.—Patient's appearance when first examined.

Examination.—The left eye-ball was completely fixed and protruded forwards and outwards. A soft mass was seen and felt in the upper and inner quadrant of the left orbit, distending the upper eye-lid in that region and appearing to be the cause of the proptosis. It was not tender and the skin moved freely over it, but the mass was fixed to the bone and neighbouring soft structures.

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The bony edges of the orbit were intact. There was a nebula on each cornea; the pupils were regular and active.

Exophthalmic Measurements: Right 22, Left 30.
Ocular Tension: Normal.
Visual Acuity: Right—Counting fingers at 2 m.
Left— Counting fingers at 1 m.
Fundi: Not easily seen because of the corneal opacities.

There was marked chronic right maxillary sinusitis associated with a large right oro-maxillary fistula; the nasal passages were free, the ethmoid regions appeared clear on both sides.

General examination revealed no abnormality. The urine and blood pictures were within normal limits. Serological tests for syphilis were normal before and after provocation.

Radiological study of the sinuses and orbits revealed:

(1) Opaque right maxillary antrum, communicating with the mouth cavity by a fistula (oro-maxillar fistula.)
(2) Generalized widening of the left orbit (Left primary orbital dilatation); the orbital margins being intact.

On exploration, the intra-orbital mass could not be freed from the neighbouring structures, and gave the impression of being malignant. A piece was removed for histo-pathological examination, and the late Prof. Bernard Shaw reported as follows:

> The histological sections, many of which have been examined, show no evidence of neoplasia. The tissues present a very unusual and unfamiliar appearance. There is a curious hyalinosis and swelling of the collagen around the blood vessels in the orbital fibro-adipose tissue (Fig. 2) and this is associated with aggregations of lymphocytes. Occasional foreign body giant cells are seen attacking the hyaline material. The appearances are those of localized amyloidosis, commonly known as " amyloid tumour ".

With this finding, I was content to treat the septic foci, leaving the mass untouched.

FIG. 2.—Photo-micrograph of biopsy. The thick-walled blood vessels show marked homogenous hyalinosis with narrowing of the lumina, due to amyloid infiltration. ×114

Comment

This most unusual finding stresses the value of routine histopathological study in intra-orbital surgery. This " tumour " could easily have been mistaken for malignant disease, and the treatment given would have meant complete loss of the left eye, which could ill be spared.

We are much indebted to the late Prof. Bernard Shaw for the care he used to take in the study of our biopsies. Our thanks are also due to Dr. Marey for his radiological study of this case.

BOOK REVIEWS

The Clinical Pathology of the Aqueous Humour. (Clinique de l'humeur aqueuse pathologique). By F. Verrey. 1954. Pp. 269, 202 figs. (27 in col.). Delachaux and Niestlé S. A., Paris and Switzerland. (*Swiss Fr.* 64; £5 15*s.* 6*d.*)

The work of the Zürich school under the direction of Marc Amsler on the chemical and pathological aspects of the aqueous humour, particularly in cases of iridocyclitis, is already well known. Diagnostic puncture of the anterior chamber had been practised sporadically for more than half a century, but only for scientific purposes and research; since 1941, Amsler has been using the method as a routine diagnostic procedure in the clinic, and the present volume, which is a résumé of some 3,000 such examinations undertaken without mishap, is a useful, interesting, and, at the same time, authoritative recapitulation of the findings. The essential investigations carred out by micro-methods on the 0·1 to 0·2 ml. of aqueous thus obtained are an estimation of the protein content of the aqueous, an enumeration and examination of its cellular content, and serological and bacteriological studies. So far as the technique itself is concerned, the author is satisfied with its ease and safety; indeed the paracentesis often has advantages such as in the assessment of the reaction of the eye to the trauma, as a guide to its probable conduct after more drastic operative procedures, in draining an inflamed eye of toxic products or in providing the opportunity of introducing therapeutic substances (penicillin, etc.) into the anterior chamber. Advanced arteriosclerosis and intra-ocular haemorrhage constitute the only contraindications. On the positive side a considerable amount of valuable information can be gleaned from the protein and crystalline content of the anterior chamber and from the nature and number of the cells present therein—a matter of diagnostic and prognostic importance. In general, in chronic inflammations, lymphocytes and tissue-monocytes do not indicate a bad prognosis unless present in large numbers, while the presence of polymorphonuclear leucocytes and bacterial phagocytes, even if few, suggests the possibility of reactivity and relapses. Bacteriological studies have been less conclusive. Occasionally a positive culture has been obtained particularly during the first 3 or 4 days of an inflammatory attack or a relapse in a chronic inflammation—mainly staphylococci and, as a rarity in severely inflamed eyes, such organisms as the tubercle bacillus and *B. subtilis*; virus culture has not been attempted. It would seem important that these studies should be pursued, since they may bring forward further information on the enigmatic subject of the aetiology of intra-ocular inflammations.

The Year Book of Eye, Ear, Nose and Throat for 1953-4. The Eye edited by Derrick Vail. The Ear, Nose and Throat edited by John R. Lindsay. 1954. The Year Book Publishers, Chicago. Pp. 455, 126 figs. (45*s.*).

This Year Book maintains the high standard of its predecessors and provides a readable and readily accessible summary of the important papers in ophthalmology which have appeared during the last year. Derrick Vail has again put the profession in his debt by the care with which he has combed the literature of our own and cognate specialties to obtain what is of interest and value, by the presentation of much diverse matter in a readily digestible form, and by the wisdom and common sense of his assessment of many of the contributions.

NOTES

The Annual Dinner of the Institute of Ophthalmology, London, will be held on Wednesday, November 24, 1954 at, 7.30 for 8 p.m. at the Royal College of Surgeons, Lincoln's Inn Fields, W.C.1. Application for tickets may be made to the Secretary, Institute of Ophthalmology, Judd Street, London, W.C.1.